THE
AMERICAN NATURALIST

VOL. LXIII *July–August, 1929* No. 687

GENETIC NOTES ON HYBRIDS OF PERENNIAL TEOSINTE AND MAIZE[1]

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GENETIC results obtained from certain hybrids of perennial teosinte (*Euchlaena perennis*) with maize (*Zea mays*) may afford some help in interpreting the cytological behavior of such hybrids. In all my cultures teosinte was the seed parent and maize the pollen parent and the F_1 hybrid plants were back crossed with maize pollen.

The following genotypes were involved, linked genes being indicated by a connecting hyphen:

Perennial Teosinte	· Maize		Maize
(*A B^w c r-G*	× *A B C R-g*)	×	*A b C r-g*
(*A r c-Wx*	× *A R C-wx*)	×	*A R c-wx*
(*Su-tu*	× *su-Tu*)	×	*su-tu*
(*y Lg*	× *Y lg*)	×	*y lg*

It will be noted that of the linked genes carried by the maize parent of the F_1 hybrid, in every instance, one was dominant and one recessive to the respective teosinte allelomorphs, and that the maize parent used in the back crosses carried only the recessive genes. It was thought that, by this arrangement, the distribution of the particular maize chromosome of the F_1 hybrids could be detected by the dominant gene of the maize parent, and that the expression of a recessive maize gene would indicate not only the presence of the maize chromosome carrying it but also the absence of any teosinte homolog.

[1] Paper No. 167, Department of Plant Breeding, Cornell University, Ithaca, New York.

Numerous difficulties were encountered in this study. The F_1 plants are only partly fertile. It was not possible, under the conditions to which the cultures were subjected, to obtain self-pollinated seeds or to get seed of maize to develop when pollinated with hybrid pollen. When the F_1 hybrids were pollinated with maize pollen not over 5 to 10 per cent. of the pistillate flowers set seed.

One of the greatest difficulties of this study came from the fact that the seeds of the F_1 hybrid of maize with perennial teosinte, unlike that of annual teosinte with maize, are embedded in the rachis and wholly covered by the rachis and thick, hard glume. It is impossible, therefore, to determine some aleurone and endosperm characters without first removing the seed from this hard covering. The small size of the seed when thus removed is an added difficulty.

RESULTS

In presenting such results as I have been able to obtain, I shall consider the several crosses in the order in which they are listed above.

$$(A\ B^w\ c\ r\text{-}G \times A\ B\ C\ R\text{-}g) \times A\ b\ C\ r\text{-}g$$

In this back cross 291 seeds were obtained from seven F_1 plants. Of these, 137 were classed as having colored aleurone and 154 as white, a deviation of 8.5 ± 5.8 from equality. The original maize parent had colored aleurone and was therefore *A C R*. The perennial teosinte with which I have worked—all my plants have come from a single seed obtained from Collins and Kempton—is, with respect to aleurone color genes, *A c r*. Since in this cross it was desired to study the *R r* pair alone of all the aleurone genes, the maize used in the back cross was *A C r*. The fact that approximately one half of the seeds were colored and one half white indicates that the maize chromosome carrying the *R* gene, or at least a part of that chromosome, was distributed to approximately one half of the functioning female gametes.

It is, of course, not impossible that, with such difficult material, some errors in classification were made. It is believed, however, that no white seeds were classed as colored and that relatively few, if any, colored seeds could have been classed as white. All the seeds, after having been removed from the bony covering, were examined carefully with a hand lens. There were grown in the greenhouse eight plants from these back-crossed seeds, four from colored and four from white seeds. These plants were all pollinated with *A C r* pollen. The four plants from colored seeds produced 301 seeds, of which 162 were colored and 139 white, a deviation of 11.5 ± 5.9 from equality. The four plants from white seeds gave a total of 168 white seeds and no colored ones.

Of the 291 seeds from the first back cross, 208 produced plants, 104 having come from colored seeds and 104 from seeds classed as white. Of the 208 plants, 196 were normal green, *G*, and twelve were golden, *g*, ten of the latter having come from colored and two from white seeds. In a similar back cross involving only pure maize, 50 per cent. of the plants are expected to be golden, but in this hybrid back cross only 5.8 per cent. were golden. It is probable, therefore, that at least one teosinte homolog, *r-G*, of the *R-g* maize chromosome was present in the great majority of the functioning female gametes of the F_1 hybrid, but that some of these gametes had no *r-G* teosinte chromosome. Some of the possibilities involved will be discussed later.

As was noted above, two of the twelve golden plants came from seeds that had been classed as white. Both these plants occurred in field-grown cultures and unfortunately did not flower early enough to make it possible to determine their aleurone-color genotypes by appropriate pollinations. If the seeds from which they grew were white, *r*, as originally classed, the two plants must have resulted from crossing over between the teosinte chromosome, *r-G*, and the maize chromosome, *R-g*. But

little value attaches to these records, both because of the small numbers involved and because the results could not be checked.

Of the 208 plants in these cultures, 100 were classed as sun red, *B*, and 108 as weak sun red, B^w. These two types of sun red are not easily distinguished in many cases, but in so far as the classification was reliable, it indicates distribution of the *B* chromosome of maize to about one half of the functioning female gametes of the F_1 hybrid.

$$(A\ r\ c\text{-}Wx \times A\ R\ C\text{-}wx) \times A\ R\ c\text{-}wx$$

Perennial teosinte, as noted earlier, has the aleurone constitution *A c r*. It has also the dominant allelomorph, *Wx*, of waxy, *wx*, endosperm. It was crossed by colored-aleurone, waxy maize. Since the *c* gene for aleurone color is the one that it was desired to study in connection with *wx*, pollen for the back cross was taken from maize plants that had the constitution *A R c-wx*.

Five F_1 plants, back crossed as indicated, produced 113 seeds, of which forty were colored to some extent and seventy-three were classed as white. If this classification were accurate, it would indicate that the *C* chromosome from the maize parent of the F_1 plants was distributed to only 35.4 per cent. of the functioning female gametes. There is reason to expect, however, that a considerable number of the seeds classed as white may have been incorrectly listed. The F_1 plants were heterozygous with respect to the *R r* pair as well as the *C c* pair. When female gametes lack *R* and are fertilized by sperm carrying *R*, the resulting aleurone is often mottled or only lightly specked with color. Some of the seeds that were classed as colored were in fact nearly white with only a few purple specks. It is not unlikely that some seeds which received *R* from the maize parent of the back cross were classed as white.

As a check on the reliability of the classification, a number of colored seeds and of seeds classed as white was planted and the resulting plants crossed with *A c R*

maize pollen. Four such back-cross plants from colored seeds gave approximately equal numbers of colored and of white seeds, sixty-one of the former to sixty-five of the latter; and one plant produced 122 colored and only twenty-six white seeds. The five seeds from which these plants grew had, therefore, been correctly listed as colored. Likewise, five plants grown from seeds classed as white were tested by pollination with *A c R* maize pollen. Four of these produced 141 white seeds and seven colored ones. The latter were presumably due to accident, the fact that in the F_1 hybrids several small ear shoots emerge at different times from the same leaf sheath making it unusually difficult to guard against accidental pollination. It is believed, therefore, that the four seeds from which these plants grew had been correctly listed as white. But one plant from a supposedly white seed yielded fifty-nine colored and fifty-two white seeds. The seed from which this plant grew was evidently genotypically colored, though listed as white.

Although this test involved too few plants to afford a means of correcting the classification of the seeds produced by the F_1 hybrids, it, nevertheless, suggests that the numbers of colored and of genotypically white seeds were more nearly equal than the records given above would indicate. At least, it would be incautious to conclude from these rather unsatisfactory records that the *C* maize chromosome was distributed to much less than half of the functioning gametes.

Of the 113 seeds from the back-crossed F_1 plants, eleven were waxy, *wx*, and 102 non-waxy, *Wx*. Although the small size of the seeds, after removal from their hard covering, made the test difficult, they were all successfully tested with iodin and the waxy nature of the eleven seeds established without question. As a further check on the classification a number of waxy and of non-waxy seeds was planted, but only one plant from waxy and two plants from non-waxy seeds were pollinated with pollen from

waxy maize. The latter plants gave only ten waxy and twelve non-waxy seeds, but the former plant, grown from a seed classed as waxy, yielded fifty-eight waxy seeds and only one non-waxy seed, the latter presumably due to accident.

Assuming that with respect to the *Wx wx* pair, the classification of the seeds of the back-crossed F_1 hybrids was entirely correct, as I believe it to have been, 9.7 per cent. of the seeds, eleven of the 113, was waxy. It is evident, therefore, in this case as in the cross involving golden, that teosinte chromosomes carrying *Wx* were distributed to many more than half of the functioning female gametes of the F_1 hybrid. But the occurrence of these waxy seeds is evidence also that a teosinte homolog, *c-Wx*, of the *C-wx* maize chromosome was not present in all functioning gametes.

Of the eleven waxy seeds, seven were classed as white and only four as purple. All the white waxy seeds were planted in order that their true genotype with respect to color might be determined. Unfortunately there resulted only one weak plant which never came into flower. It seems unlikely that all these seven seeds were genotypically white, for this would indicate among the waxy seeds a high cross-over percentage between the *c-Wx* teosinte and the *C-wx* maize chromosomes. Evidently this material is not satisfactory for testing crossing over.

$$(Su\text{-}tu \times su\text{-}Tu) \times su\text{-}tu$$

Perennial teosinte, with starchy endosperm and normal glumes, *Su-tu*, was crossed by sugary tunicate maize, *su-Tu*, and two F_1 hybrid plants were back crossed by sugary normal maize, *su-tu*. There resulted fifty-eight seeds, fifty-four of which were classed as starchy, *Su*, and four as sugary, *su*. It is quite impossible to be certain of the correctness of such a classification when dealing with seeds so small as those of this F_1 hybrid. The seeds classed as sugary were, however, somewhat wrinkled and when cut showed the characteristic glassy appearance of

sugary endosperm. The four seeds classed as sugary were planted, but only two of the resulting plants produced silks. These, when pollinated by sugary maize, produced 137 sugary and two starchy seeds, the latter presumably accidents. Three plants from supposedly starchy seeds were tested in the same way. Two of them yielded fifty-three starchy and fifty-one sugary seeds, indicating that the seeds from which they grew had been correctly classified. The third plant, however, produced twenty-six sugary and no starchy seeds. There can be no doubt, therefore, that at least three sugary seeds were produced by the back-crossed F_1 plants and there were presumably at least five, perhaps more than five, such seeds. If there were five, and only five, sugary seeds, 9.6 per cent. of the functioning female gametes could not have had present any teosinte homolog, *Su-tu,* of the *su-Tu* maize chromosome.

From the fifty-eight seeds of the back-crossed F_1 plants, there were grown fifty-one plants, twenty-three of which were tunicate and twenty-eight normal. The *Tu* maize chromosome was, therefore, distributed to 45.1 per cent. of the functioning female gametes of the F_1 hybrid, the deviation from 50 per cent. being only 4.9 ± 2.4. Four of the tunicate plants came from sugary seeds, and no seed classed as sugary produced a normal plant. There was, therefore, no indication of crossing over between the *Su-tu* teosinte chromosome and the *su-Tu* maize chromosome.

$$(y\ Lg \times Y\ lg) \times y\ lg$$

Perennial teosinte has white endosperm, *y,* and normal leaves, *Lg.* It was pollinated by maize with yellow endosperm, *Y,* and liguleless leaves, *lg.* Nine F_1 plants were back-crossed by maize with white endosperm and liguleless leaves. The back-crossed F_1 plants produced seventy-nine seeds, of which forty-one were classed as having yellow and thirty-eight as having white endo-

sperm. Only one plant from yellow seed and two from seeds classed as white were tested by crossing with pollen from white endosperm maize. One of the plants from supposedly white seeds produced twenty-five white seeds and three yellow ones, the latter thought to be due to accidental pollination. The other plant, from a seed classed as white, gave ten yellow and thirteen white seeds. The plant from a seed that had been classed as yellow gave forty-two yellow and forty-five white seeds. These tests show that at least one error of classification with respect to endosperm color of the seeds of the back-crossed F_1 plants was made. Whether other errors were made is not known. It is certain, however, that not far from one half of the functioning female gametes of the F_1 hybrids had the *Y* maize chromosome.

Only twenty-five plants were grown from seeds of the back-crossed F_1 plants. Of these, only one, or 4 per cent., had liguleless leaves. Evidently the female gametes from which this plant arose lacked a teosinte homolog, *Lg*, of the *lg* maize chromosome.

SUMMARY AND DISCUSSION

With respect to the genes concerned in these tests, perennial teosinte has the constitution *B^{pe}-Lg c-Wx r-G Su-tu y*. It was pollinated by four strains of maize involving the genes *B-lg C-wx R-g su-Tu Y*, and the F_1 plants were back-crossed with pollen of four strains of maize carrying the corresponding recessives *b-lg c-wx r-g su-tu y*. When an aleurone-color gene was involved, the maize plants used in back crossing carried dominant aleurone-color genes except for the one concerned in that cross.

The dominant characters exhibited by the maize parents and the corresponding recessives carried by perennial teosinte appeared, with one marked exception, in approximately one half of the seeds or plants resulting from the back crosses, as follows:

Number of seeds or plants				
Recessive teosinte genes		Dominant maize genes	Total	Deviation from one half dominant
B^w	108	B 100	208	(−) 4.0 ± 4.9
r	154	R 137	291	(−) 8.5 ± 5.8
tu	28	Tu 23	51	(−) 2.5 ± 2.4
y	38	Y 41	79	(+) 1.5 ± 3.0
c	73	C 40	113	(−) 16.5 ± 3.6
Total	401	341	742	(−) 30 ± 9.2
Total exclusive of $C\,c$	328	301	629	(−) 13.5 ± 8.5

Great difficulty was experienced in classifying seeds involving the $C\,c$ pair. Many of the seeds classed as colored were white except for a few specks of color, and some classed as white, c, may well have been genotypically colored, C; one plant, of five tested from seeds classed as white, proved to be $C\,c$. Some difficulty was experienced also in classifying seeds involving $Y\,y$ and plants involving $B\,B^w$, and appropriate tests showed that at least one seed classed as white, y, was genotypically yellow, $Y\,y$. On the whole, however, except for $C\,c$, it is believed that the classification was substantially correct. For the four maize chromosomes carrying the dominant genes B, R, Tu and Y, it can be said that they were distributed to approximately one half of the functioning female gametes of the F_1 hybrids. There were in general slightly fewer individuals with the maize than with the teosinte chromosome, but in no case, except that involving $C\,c$, was the deviation from one half statistically significant. The genetic behavior of these F_1 maize-teosinte hybrids with respect to dominant maize characters was, in short, substantially the same as that of pure maize crosses. It is evident, therefore, that few if any maize chromosomes are lost during the reduction divisions by which the functional megaspores are formed.

With respect to the recessive maize-parent characters of these maize-teosinte hybrids, the genetic results were

quite unlike those obtained with pure maize. In such back crosses with pure maize, the recessive characters of the parents, as well as the dominant ones, ordinarily appear in approximately one half of the progeny. In no instance, however, was that true of these maize-teosinte hybrids. The results are as follows:

Number of seeds or plants				
Dominant teosinte genes		Recessive maize genes	Total	Percentage recessive characters
G	196	*g* 12	208	5.8
Lg	24	*lg* 1	25	4.0
Su	53	*su* 5	58	8.6
Wx	102	*wx* 11	113	9.7
Total	**375**	**29**	**404**	**7.2**

It was quite impossible to be certain of the correctness of the classification with respect to the pair *Su su*, and appropriate tests showed that at least one error had been made. There is no doubt of the correctness of the classification of the other characters involved. Instead of 50 per cent. of the back-cross seeds and plants showing the recessive characters of the maize parent of the F_1 hybrids, as would be expected in pure maize crosses, the observed percentages ranged from 4.0 to 9.7, with an average of 7.2.

Maize normally has twenty chromosomes, ten pairs, while perennial teosinte has forty chromosomes, twenty pairs according to Longley,[2] more commonly ten groups of four each according to Randolph (unpublished figures). Both Longley and Randolph agree that the F_1 of perennial teosinte-maize hybrids shows numerous trivalents together with bivalents and univalents. The trivalents have not infrequently one chromosome loosely attached to the other two. Longley reports irregular heterotypic divisions with lagging chromosomes, particu-

[2] A. E. Longley, "Chromosomes in Maize and Maize Relatives," *Journ. Agr. Research*, 28: 673–681, 1924.

larly univalents, some of which are not included in either daughter nucleus, and suggests that it is largely the maize chromosomes which thus lag and fail to be included in the daughter nuclei.

If, in the triploid F_1 of hybrids of maize with perennial teosinte, any three homologous chromosomes disjoin and assort at random, two going to one pole and one to the other pole, 50 per cent. of the gametes should have the one chromosome from the maize parent, as the genetic evidence indicates, and 16.7 per cent. of the gametes should lack both teosinte homologs. If this were true of the functioning female gametes of the F_1 hybrid, 16.7 per cent. of the individuals of the back-cross progenies should exhibit the recessive character of the maize parent. But only about 7 per cent. of the individuals had such recessive characters. It seems likely, therefore, that trisomic disjunction is not wholly at random, but that the two teosinte chromosomes more commonly disjoin and pass one to either pole, while the maize chromosome is distributed at random.

If, however, the two teosinte chromosomes always disjoined, one always going to one pole and the other to the other one, every gamete must have one teosinte homolog. And, since this chromosome carried, in these tests, the dominant allelomorph of a maize gene, recessive maize characters could appear in none of the back-cross progenies. Apparently, at least so far as the functioning female gametes of the F_1 hybrids are concerned, some behavior between the two extremes of random disjunction of trisomic groups on the one hand and of universal disjunction of teosinte bivalents on the other must be considered.

Another possible explanation of the occurrence of less than the 16.7 per cent. of recessives among the back-cross progenies expected on the basis of random disjunction of trisomic teosinte-maize groups must be noted. If any considerable number of the maize chromosomes were to

lag on the spindle and not be included in the daughter nuclei, as suggested by Longley, less than 16.7 per cent. of recessives might appear in the progeny of the F_1 hybrids. But, if the maize chromosome were not included in the daughter nuclei in any considerable number of cases, the dominant maize character carried by it could not appear in approximately half of the back-cross progeny. Moreover, only univalents would be expected to behave in this way, and to say that the maize chromosome may be univalent and the two teosinte chromosomes bivalent more commonly than expected on random disjunction is equivalent to saying that the disjunction is not wholly a random one.

In conclusion, it seems safe to assume that, in F_1 maize-teosinte hybrids, the maize chromosome of any group is distributed to about half of the functioning female gametes and that a teosinte homolog is present in more than five sixths of these gametes—in short, that the maize chromosomes are distributed at random and that the teosinte chromosomes are not wholly so distributed.

INHERITANCE OF SEMI-STERILITY IN MAIZE[1]

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A HERITABLE type of partial sterility involving abortion of one half the pollen grains and embryo sacs has recently been reported in maize (Brink, 1927). A similar condition in the Velvet bean (Stizolobium) has been described by Belling (1914), and Blakeslee and Cartledge (1926) have found it in Datura. Belling called the phenomenon semi-sterility, and this name is being retained. The chance discovery of semi-sterility in these three widely different plants which happen to have come under the purview of the geneticist suggests that the phenomenon may be of wide occurrence. As one of the mechanisms by which interspecific sterility may arise it deserves careful analysis.

IDENTIFICATION OF SEMI-STERILE PLANTS

Semi-sterile maize plants are recognizably different from normal plants only in fertility. Fifty per cent. of the pollen is abortive, and the ears produced bear one half the normal number of seeds. We find that microscopic examination of the pollen mounted in iodine solution is the most accurate and convenient method of classification. The aborted grains from semi-sterile plants do not contain reserve food materials and stand out clearly in the reagent from the deeply staining starch-rich normal type. Bursting of the sound pollen grains in the iodine solution does not occur if the anther is previously killed with formalin-alcohol. Classification on the basis of seed development is not as satisfactory. The ears on semi-sterile plants are never more than half filled, but incomplete pollination, disease and other causes sometimes produce a similar condition on normal plants.

[1] Paper from the department of genetics, Agricultural Experiment Station, University of Wisconsin, No. 97. Published with the approval of the director of the station.

BREEDING BEHAVIOR

The early results on the inheritance of semi-sterility in maize indicated that reciprocal crosses between normal and semi-sterile plants give equal numbers of these two classes among the offspring. Self-pollination of semi-sterile individuals gives a like result (Brink, 1927). More extensive data on these relations are now available.

SEMI-STERILE ♀ × NORMAL ♂ CROSSES

In Table I the results obtained in 1927 in eighteen progenies from crosses of the type, semi-sterile ♀ × normal ♂, are presented. Excepting in progenies R188 and R189, the distributions from which are combined in the last entry, approximately equal numbers of the two

TABLE I

DISTRIBUTION OF SEMI-STERILE AND NORMAL PLANTS FOLLOWING MATINGS OF THE TYPE, SEMI-STERILE ♀ × NORMAL ♂. 1927 RESULTS

Progeny Number	Parents	Number of plants			Deviation on 1:1 basis	Dev. / P.E.
		Total	Semi-Sterile	Normal		
R171	R160–39 × R141–18	36	17	19	1 ± 2.0	0.5
R172	R160–7 × R141c–8	23	14	9	2.5 ± 1.6	1.6
R173	R160a–5 × R141a–8	34	23	11	6 ± 2.0	3.0
R174	R160a–24 × S45–3	58	33	25	4 ± 2.6	1.5
R175	R160–9 × S45–13	91	44	47	1.5 ± 3.2	0.5
R176	R160–41 × R112–18	56	28	28	0 ± 2.5	.....
R177	R160–63 × G.B.–3	37	22	15	3.5 ± 2.1	1.7
R178	R160–45 × G.B.–1	48	27	21	3 ± 2.3	1.1
R179	R160a–9 × R86b–24	45	19	26	3.5 ± 2.3	1.5
R180	R160–25 × L20–4	65	31	34	1.5 ± 2.7	0.6
R181	R160–5 × L20–7	68	31	37	3 ± 2.8	1.1
R182	R160–37 × L26–1	62	28	34	3 ± 2.7	1.1
R183	R160–22 × L26–7	57	34	23	5.5 ± 2.6	2.1
R184	R160–33 × L22–7	22	12	10	1 ± 1.6	0.1
R185	R160–40 × L22–1	32	18	14	2 ± 1.9	1.0
R186	R160a–23 × L24–8	18	9	9	0 ± 1.4	.....
R187	R160–1 × L24–5	24	14	10	2 ± 1.7	1.2
R188, R189	R160–30 × A7ay–29	39	9	30	10.5 ± 2.1	5.0
Totals		**815**	**413**	**402**	**5.5 ± 9.6**	**0.6**

classes of offspring occur in each family. The irregular behavior of R188 and R189 is being studied further.

In 1928 twenty-nine additional progenies from the same type of mating were examined. The results are given in Table II. With the possible exception of family C8 the

TABLE II

DISTRIBUTION OF SEMI-STERILE AND NORMAL PLANTS FOLLOWING MATINGS OF THE TYPE, SEMI-STERILE ♀ × NORMAL ♂. 1928 RESULTS

Progeny Number	Parents	Number of plants			Deviation on 1 : 1 basis	Dev. / P. E.
		Total	Semi-Sterile	Normal		
C1	R168–5 × –75	78	39	39	0.0 ± 0.0	.....
C2	–89 × –72	54	33	21	6.0 ± 2.5	2.4
C3	R169–27 × –2	44	25	19	3.0 ± 2.2	1.4
C4	–26 × –10	53	29	24	2.5 ± 2.5	1.0
C5	R173–1 × –3	63	30	33	1.5 ± 2.7	0.6
C6	R174–23 × –20	74	35	39	2.0 ± 2.9	0.7
C7	R180–3 × –2	22	11	11	0.0 ± 1.6	.....
C8	–35 × –33	66	24	42	9.0 ± 2.7	3.3
C9	R185–4 × –5	83	50	33	8.5 ± 3.1	2.7
Sub-totals		537	276	261	7.5 ± 7.8	1.0
R321	R169–31 × L54–5	145	71	74	1.5 ± 4.1	0.4
R322	R169–38 × L54–12	128	69	59	5.0 ± 3.8	1.3
R323	R169–3 × L54–5	127	73	54	9.5 ± 3.8	2.5
R324, a	R169–7 × L54–9	88	42	46	2.0 ± 3.2	0.6
R325, a	R169–36 × L54–5	154	83	71	6.0 ± 4.2	1.4
R326	R169–15 × L54–8	188	106	82	12.0 ± 4.6	2.6
R327, a	R169–9 × L54–8	104	60	44	8.0 ± 3.4	2.3
R328	R171–36 × R197–9	115	66	49	8.5 ± 3.6	2.4
R329, a, b, c	R171–1 × R197–12	92	44	48	2.0 ± 3.2	0.6
R330, a, b, c	R171–10 × R197–14	121	61	60	0.5 ± 3.7	0.1
R331, a, b, c	R171–6 × R197–1	102	45	57	6.0 ± 3.4	1.8
R332, a, b, c	R171–36 × R197–1	181	88	93	2.5 ± 4.5	0.6
R335, a	R172–19 × R201a–2	108	53	55	1.0 ± 3.5	0.3
R337, a	R172–3 × R201a–2	94	40	54	7.0 ± 3.3	2.1
R338, a, b	R176–56 × L89b–5	210	114	96	9.0 ± 4.9	1.8
R342, a	R177–31 × R270–1	173	78	95	8.5 ± 4.4	1.9
R343, a, b, c	R178–24 × R197–12	92	43	49	3.0 ± 3.2	0.9
R348, a	R168–116 × R196–4	105	52	53	0.5 ± 3.5	0.1
R351, a	R183–57 × R197–9	82	32	50	9.0 ± 3.1	2.9
R352, a	R183–50 × R196–8	172	84	88	2.0 ± 4.4	0.4
Sub-totals		2,581	1,304	1,277	13.5 ± 17.1	0.8
Totals		**3,118**	**1,565**	**1,553**	**6.0 ± 18.8**	**0.3**

deviations from equality in the two classes are within the limits of random sampling.

NORMAL ♀ × SEMI-STERILE ♂ CROSSES

When a semi-sterile plant is used as the staminate parent in a cross with the normal type the same result is obtained as in the mating described above. In Table III

TABLE III

DISTRIBUTION OF SEMI-STERILE AND NORMAL PLANTS FOLLOWING MATINGS OF THE TYPE, NORMAL ♀ × SEMI-STERILE ♂. 1927 RESULTS

Progeny Number	Parents	Number of plants			Deviation on 1 : 1 basis	Dev. / P. E.
		Total	Semi-Sterile	Normal		
R163 } R163a }	R160–81 × –47	255	136	119	8.5 ± 5.4	1.6
R164 } R164a }	R160–61 × –41	227	130	97	16.5 ± 5.1	3.2
Totals		482	266	216	25.0 ± 7.4	3.4

the results of two combinations of this type tested in 1927 are shown. It will be noted that in one of the crosses a possibly significant deficiency of normals occurs.

Further data of the same sort obtained in 1928 are presented in Table IV. In ten of the progenies the distributions are in close agreement with expectation on the 1:1 basis; in the eleventh, a probably significant excess of normals is shown.

In general, it is clear that matings between normal and semi-sterile plants, either way the cross is made, result in equal numbers of the two sorts of offspring. The occasional occurrence of progenies deviating rather widely from this proportion requires further investigation.

SELF-POLLINATION OF SEMI-STERILE PLANTS

In Table V the distributions of normal and semi-sterile plants in seventeen progenies from self-pollinated semi-sterile plants are given. Again we find approximately

TABLE IV

DISTRIBUTION OF SEMI-STERILE AND NORMAL PLANTS FOLLOWING MATINGS OF THE TYPE, NORMAL ♀ × SEMI-STERILE ♂. 1928 RESULTS

Progeny Number	Parents	Number of plants			Deviation on 1 : 1 basis	Dev. / P. E.
		Total	Semi-Sterile	Normal		
C10	R168–102 × –116	272	131	141	5.0 ± 5.6	0.9
C11	–72 × –89	175	83	92	4.5 ± 4.5	1.0
C12	–87 × –112	128	60	68	4.0 ± 3.8	1.0
C14	–126 × –127	132	71	61	5.0 ± 3.9	1.3
C15	R169–2 × –27	32	18	14	2.0 ± 1.9	1.0
C16	–10 × –26	11	5	6	0.5 ± 1.1	0.4
C17	R180–19 × –15	132	68	64	2.0 ± 3.9	0.5
C18	R181–24 × –3	39	20	19	0.5 ± 2.1	0.2
C19	R182–9 × –11	90	33	57	12.0 ± 3.2	3.7
C20	–25 × –29	109	57	52	2.5 ± 3.5	0.7
R373*		198	99	99	0.0 ± 4.8	.....
Totals		**1,318**	**673**	**645**	**14.0 ±12.2**	**1.1**

* R373 was segregating for tassel-seed (ts_2). Only the Ts_2 plants were classified for semi-sterility.

TABLE V

DISTRIBUTION OF SEMI-STERILE AND NORMAL PLANTS AMONG THE OFFSPRING OF SELF-POLLINATED SEMI-STERILE PLANTS

Progeny Number	Parents	Number of plants			Deviation on 1 : 1 basis	Dev. / P. E.
		Total	Semi-Sterile	Normal		
C21	R168–5	53	27	26	0.5 ± 2.5	0.2
C22**	–11	95	45	50	2.5 ± 3.3	0.8
C23**	–21	18	13	5	4.0 ± 1.4	2.9
C24	–64	22	14	8	3.0 ± 1.6	1.9
C25	–69	23	9	14	2.5 ± 1.6	1.6
C26	–127	99	52	47	2.5 ± 3.4	0.7
C27	R169–15	65	35	30	2.5 ± 2.7	0.9
C28	–20	20	11	9	1.0 ± 1.5	0.7
C29	–24	37	22	15	3.5 ± 2.0	1.7
C30	–58	22	17	5	6.0 ± 1.6	3.7
C31	–81	22	13	9	2.0 ± 1.6	1.2
C32	R181–1	38	20	18	1.0 ± 2.1	0.5
C33	–3	18	8	10	1.0 ± 1.4	0.7
C34	R182–33	12	6	6	0.0 ± 1.2	.....
C35	–41	26	17	9	4.0 ± 1.7	2.4
C36	–46	22	11	11	0.0 ± 1.6	.....
C37	–60	48	22	26	2.0 ± 2.3	0.9
Totals		**640**	**342**	**298**	**22.0 ± 8.5**	**2.6**

** Segregating for tassel-seed; only *Ts* plants classified.

equal numbers of the two classes of offspring. Families C23 and C30, however, show deficiencies in the normal group which may not have resulted wholly from chance. The deviations in these two progenies account for about one half that found in the totals.

PROVISIONAL EXPLANATION

In interpreting these results we must keep in mind the conditions under which the semi-sterility arose. A satisfactory explanation must account not only for the breeding facts but for the origin of semi-sterility as well.

Semi-sterility appeared in about 9 per cent. of the offspring of a self-pollinated plant belonging to a line definitely known to be normal in the preceding generation. Apparently the change occurred in a vegetative cell from which a portion of the sporogenous tissue was descended.

As was pointed out in a previous paper (Brink, 1927) the pedigree culture data may be satisfactorily interpreted on a dihybrid basis. It might be assumed, for example, that semi-sterile plants are AaBb in composition and that the AB and ab classes of spores abort. On this hypothesis self-pollination or backcrossing to the normal type, whether AAbb or aaBB, would give equal numbers of semi-sterile and normal plants. An AaBb plant, however, could arise only from either AAbb or aaBB individuals by simultaneous mutations in two different loci. While this is not impossible it is highly improbable in view of what we know concerning the rate of gene mutation.

It seems more likely that a change of some sort has occurred in one chromosome that is compensated for by alteration of another member of the set. One might assume that a section of one chromosome carrying genes active in the gametophyte, or an entire chromosome, has become attached to a non-homologous member of the complement. If the modified and normal chromosomes assort at random in the reduction divisions two classes

of balanced and two classes of unbalanced spores will be formed in a 1:1:1:1 ratio. One half the unbalanced spores receive the translocated section (or whole chromosome) in duplicate and the remainder lack it entirely. It is assumed that both these types abort. This hypothesis is illustrated diagrammatically in Figure 1.

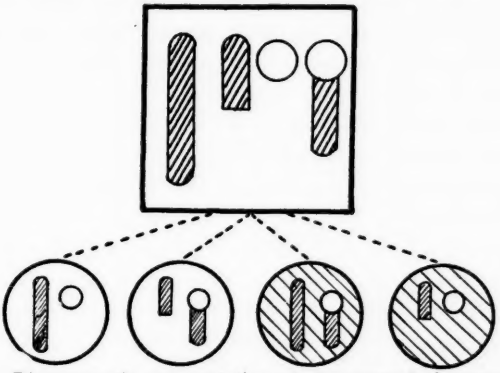

FIG. 1. Diagrammatic representation of the assumed chromosomal condition in semi-sterile maize. Two pairs of chromosomes are involved, illustrated as rods and circles, respectively. In a semi-sterile plant, as represented by the square, a piece of one chromosome is transferred to a non-homologous chromosome and assorts with it. The circles below represent the expected, equally frequent classes of spores. The two types at the right, which are cross-hatched, are assumed to abort; the other two are functional.

In accounting for similar breeding facts obtained in his studies on semi-sterility in Stizolobium, Belling (1925) suggests that an interchange of materials has occurred between two non-homologous chromosomes. While this hypothesis fits the facts in maize satisfactorily our explanation on the basis of a transference of a part of one chromosome to another is simpler and conforms equally well to the experimental results.

OCCURRENCE OF A NEW CLASS OF NORMAL PLANTS AMONG THE OFFSPRING OF SEMI-STERILE INDIVIDUALS

According to the scheme outlined above two types of fully fertile plants should occur among the offspring of

semi-sterile individuals. One of these is identical with the normal stock from which semi-sterility arose, and the other is homozygous for the translocation. Each of the types is fertile with its own kind, but the two, when crossed, give semi-sterile offspring. We have designated the new class of fertile plants, x-normal. In contrast with it the original type may be termed o-normal.

Normal sib plants in progenies resulting from matings between semi-sterile and normal individuals should all be of one type, either o-normal or x-normal, depending on the class to which the fertile parent belonged. The fully fertile offspring of self-pollinated semi-sterile plants, on the other hand, should consist of the two classes, o-normal and x-normal, in equal numbers. The results of experiments designed to test these relations are discussed in the following paragraphs.

Ten crosses were made in 1927 between pairs of normal plants in two progenies resulting from normal ♀ × semi-sterile ♂ combinations. The offspring were grown in 1928 and the pollen from eight to fourteen plants in each family examined. If no semi-sterile individuals are found in samples of this size it may be concluded with reasonable assurance that the progenies represented include only normal plants. All the plants tested in the ten families were normal.

Similarly fourteen families resulting from crosses between normal sibs in progenies coming from two matings of the reciprocal type, namely, semi-sterile ♀ × normal ♂, were tested. Thirteen of the families contained only normal plants, as expected, and one showed eight normals and two semi-steriles. The exceptional case is probably due to contamination of the parent ear by foreign pollen.

In Table VI the results are shown of 109 crosses between normal individuals in three progenies resulting from self-pollination of semi-sterile plants. It is expected that one half the crosses will give all semi-sterile

TABLE VI

RESULTS OF CROSSES BETWEEN NORMAL SEGREGATES IN PROGENIES FROM SELF-POLLINATED SEMI-STERILE PLANTS

	Number of Progenies		
	All semi-sterile	All normal	Total
Observed	54	55	109
Expected on 1:1 basis	54.5	54.5	109

offspring and the remainder all normal offspring. Fifty-four families of the first type were found and fifty-five of the latter.

These facts afford decisive evidence for the occurrence of a new class of plants, which we have called x-normal, among the offspring of semi-sterile individuals. It is fully fertile with its own kind but gives all semi-sterile offspring when crossed with the original normal strain. Presumably, o-normals and x-normals are of identical genic composition. The difference between them consists in the chromosomal relationship of a particular group of linked factors. According to our hypothesis this set of genes is transferred from one chromosome to a non-homologous chromosome in x-normal plants. The assumed chromosomal relations in o-normal and in x-normal plants are illustrated diagrammatically in Figure 2.

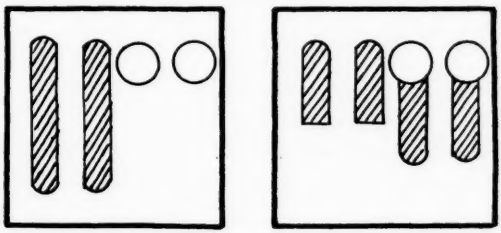

FIG. 2. Diagrammatic representation of an o-normal plant (on the left) and an x-normal plant (on the right). The latter type is homozygous for the translocation and is fully fertile.

DISCUSSION

In its breeding relations semi-sterility in maize is similar to the case described by Belling (1914) in Stizo-

lobium and one of the types of sterility Blakeslee and Cartledge (1926) find in Datura. It is not improbable that the phenomenon as manifested in the three forms is due to the same type of change in the hereditary mechanism.

Belling (1914) found that in hybrids between the fully fertile Florida Velvet bean and three other normal strains of Stizolobium, namely, Lyon, Yokohama and China, one half of the pollen grains and ovules aborted at an early stage. Semi-sterile plants, when allowed to self-pollinate, produced two classes of offspring, normal and semi-sterile, in a ratio of 1:1. In subsequent generations the normals bred true and the semi-sterile plants continued to throw equal numbers of normals and semi-steriles. The results were interpreted on a dihybrid basis. It was assumed that the Florida Velvet bean was of the composition *KKll*, and the other three stocks, which gave semi-sterile offspring when crossed with it, *kkLL*. The presence of either *K* or *L*, but not both, was considered essential for the production of a viable spore. *KL* and *kl* microspores and macrospores, consequently, abort; the *Kl* and *kL* types, corresponding to those produced by the fertile parental forms, develop into functional gametophytes.

More recently Belling (1925) has reinterpreted these facts in accordance with his view that semi-sterility is due to the interchange of segments between two non-homologous chromosomes.

In the brief report made on semi-sterility in Datura, Blakeslee and Cartledge (1926) favor the Belling hypothesis. Basing their results on tests with the different Primary $(2n+1)$ types, these workers find that in one race which gives semi-sterile offspring in certain combinations the Echinus and Microcarpic chromosomes are altered. In another line distinct from the former the exchange of chromatin appears to have taken place between the Globe and Cocklebur members of the com-

plement. In this case, however, the hybrid shows 25 per cent. pollen abortion (Blakeslee and Cartledge, 1927).

The hypothesis offered to account for semi-sterility in Datura is intimately bound up with the interpretation of the cytological behavior of certain heteroploids in this form. Belling and Blakeslee (1923) find that at the late prophase and metaphase of the first division in the pollen-mother-cells in diploid, triploid and tetraploid Daturas homologous chromosomes are connected by their ends. In certain 25-chromosome mutants, attachment may be found between two non-homologous members of the complement (Belling and Blakeslee, 1925).

Wiry, a $(2n + 1)$ Tertiary with three chromosomes belonging to set IX, may be taken as an example. In this form one of the three number IX chromosomes is often attached to one of the large chromosomes of bivalent I. Belling and Blakeslee (1925) assume that since the altered chromosome in Wiry unites both with I and with IX it has interchanged a terminal segment with chromosome I. In other words, one end is normal, attracting its fellow of set IX, while the other end carries a segment homologous with and attracted by one of the ends of chromosome I.

It will be noted that Belling and Blakeslee rest their hypothesis of segmental interchange upon the observed fact of attachment between non-homologous chromosomes. One should expect, therefore, if semi-sterility is due to segmental interchange, as Belling and Blakeslee contend, that attachment between non-homologous chromosomes would occur in plants of this class. Blakeslee and Cartledge (1926) state, however, that such attachment has not been found in F_1 individuals showing pollen abortion. On the other hand, it occurs freely in another cross not showing defective pollen. This apparent inconsistency in behavior throws some doubt upon the adequacy of the interpretation Belling and Blakeslee have put forward to account for semi-sterility and related phenomena in Datura.

Bridges (1923) has described a condition in Drosophila which appears similar in some respects to semi-sterility in plants. A piece at the right end of the second-chromosome, eight units in length, is detached from its customary place and joined to the third chromosome at a point to the left of rough (locus 86.5). Apparently all the gametes formed, including the unbalanced types, are functional. Among the zygotes, however, the deficiency in single dose is lethal. When the translocated section is present in triplicate the flies are only slightly lower in viability than normal. Individuals possessing four doses of the section in question die.

Among the offspring of X-rayed Drosophila, Muller (1928) finds many cases of translocation. Attachments between one long autosome and another and between either long autosome and the X or the Y have been observed. One case of double translocation involving all three long chromosomes was found. According to Muller there appears to be little or no preference for attachment between particular chromosomes except that the likelihood of a chromosome serving as a place of attachment for a fragment of another varies roughly with the length of the chromosome. Unbalanced combinations of chromatin appear to be transmitted through the gametes of the X-rayed Drosophila, but the zygotes receiving them frequently fail to survive.

A crucial test of the hypothesis we have outlined to account for semi-sterility in maize is not yet possible. Experiments are in progress to determine by means of linkage tests with factors whose positions are known the particular chromosomes which are modified in semi-sterile plants. Although the delimitation of certain linkage groups in maize is still in doubt and the number of known genes on several chromosomes very small it may be possible to locate roughly the point in the chromosome at which the break has occurred and the new position of the translocated piece. We should expect, if our ex-

planation is correct, that a group of genes normally in one group will show linkage with those on a distinct chromosome. The factors in the translocated piece may fail to show crossing-over among themselves, although Muller (1928) finds that in the offspring of X-rayed Drosophila the translocated piece sometimes crosses over with its normal homologue that is differently placed. The frequency of crossing-over in these flies, however, is usually much disturbed.

We have assumed that one of the abortive classes of spores produced by semi-sterile plants lacks a section of a particular chromosome and that the other non-functional class carries it in duplicate. The criticism might be made that the addition of a chromosome or a piece of a chromosome to the normal complement seldom so upsets the genic balance that the modified spore dies. It is known, for example, that $(n + 1)$ gametophytes are functional in Datura and Oenothera. Apparently $(n - 1)$ spores, on the other hand, do not develop, since $(2n - 1)$ zygotes have not been found. This is a relative matter, however, and probably dependent in large degree on the particular genes concerned. Addition of certain factors to the normal haploid complement might lead to abortion whereas the presence of others in double dose would not.

Since semi-sterile plants produce equal numbers of functional and abortive spores the modified chromosomes must assort at random in the reduction divisions. It is difficult to see why this should take place if the phenomenon is due to attachment of two entire non-homologous chromosomes end-to-end. We might expect in such a case that one half the spores would receive the attached chromosomes and the other half the two free members. Both classes would then carry a normal complement of factors and be functional. A similar distribution is conceivable with translocation of a piece of one chromosome to the end of another. Attachment at some

point other than at the ends is a more likely explanation. Such an arrangement would probably interfere with conjugation between the transferred piece and the normally placed homologous section.

Semi-sterility is of interest from the evolutionary standpoint in that it provides a means of establishing from a common ancestral stock two or more intra-fertile lines which are partially sterile with each other. One change of the type found in semi-sterile maize results in the formation of one new class of plants 50 per cent. sterile with the normal type. With two changes of this kind the production of a stock 75 per cent. sterile with the original form can occur; with three, 87.5 per cent. and so on. A fully fertile type nearly 97 per cent. sterile with the original form is possible with five translocations.

If semi-sterility arises in a small proportion of the individuals in a population breeding at random it will not persist. Assuming equal productivity in the two fertile types, o-normal and x-normal, and a 50 per cent. handicap on semi-sterile individuals, it may be shown that the proportion of x-normals will decrease in each generation. If the proportion of o-normals to x-normals and semi-steriles is large most of the individuals in the latter classes will be mated with o-normals. The result of such combination will be that the x-normals will produce semi-sterile offspring whereas the fertile segregates from the semi-sterile plants will be of the o-normal type. So in each succeeding generation the x-normals tend to be shifted into the semi-sterile class and the semi-steriles into the o-normal group. The eventual result is a practically complete return of the population to its original condition.

In a population reproducing by self-fertilization, on the other hand, the course of events is different. The fertile offspring of semi-sterile plants will be divided equally in each generation between the o-normal and x-normal classes and the latter groups, of course, will produce only their own kind. Only one half the offspring of semi-

sterile plants in each generation will be semi-sterile. As long as x-normals are in the minority the rate of gain of this class will be higher than that of the o-normal group. The tendency is towards elimination of semi-sterile individuals and the production of equal numbers of the two fertile classes. Attainment of an equal proportion of o-normals and x-normals, however, while theoretically possible, will be exceedingly slow after the first few generations, particularly if the proportion of semi-fertile plants is low at the start. This follows from two circumstances. First, the semi-sterile group from which the additional x-normals (and also o-normals) are derived is being halved in each generation; and, secondly, the relative change in proportion of o-normals and x-normals through gains from the semi-sterile class becomes less and less as the equilibrium point is approached. Relatively speaking, therefore, the population may become stabilized far short of the position theoretically attainable.

Summary

(1) Reciprocal crosses between normal and semi-sterile maize plants give equal numbers of normal and semi-sterile offspring. Self-pollination of semi-sterile individuals likewise produces the same two classes in the same proportion.

(2) In explanation of these results it is suggested that a section of one chromosome carrying genes active in the gametophyte, or an entire chromosome, has become attached to a non-homologous member of the complement. The modified and normal chromosomes assort at random in the reduction divisions. Spores receiving the translocated section in duplicate or lacking it entirely abort. The other two classes are functional.

(3) The existence of a new class of normal plants among the offspring of semi-sterile individuals has been definitely established. These plants, called x-normals,

are fully fertile and give all semi-steriles when crossed with the original normal type.

(4) If semi-sterility arises in a small proportion of the individuals in a population breeding at random the population will return quickly to its original condition. In a self-pollinated species, however, x-normal plants will become established and may eventually constitute 50 per cent. of the population, although attainment of this proportion is extremely slow after the first few generations.

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LINKAGE OF QUALITATIVE AND QUANTITATIVE GENES IN MAIZE[1]

PROFESSOR E. W. LINDSTROM

SEVEN years ago I commenced a series of investigations to test for the presence of genes for quantitative characters in the known linkage groups of two species, one a cross-fertilized type, *Zea mays,* and the other a naturally self-fertilized species, *Lycopersicum esculentum.* In the latter I have already reported (1924, 1926 and 1928) that genes controlling fruit size of the tomato show genetic linkage with qualitative genes on the first three chromosomes of that species. In maize, a great mass of data bearing on a similar situation has been collected. Since this will necessitate publication in several long reports, it seems well to present a brief summary of the results, giving a general picture of the relations discovered.

Number of rows on the maize ear was chosen as a typical quantitative character (East, 1910; and Emerson and East, 1913). A suspicion that some genetic correlation might exist between row number and cob color, for example, was aroused by the fact that all known eight-rowed varieties of maize have the white-cob, none are red-cobbed. Accordingly cob color and other qualitative characters, such as aleurone and endosperm color as well as endosperm texture, were brought into the problem.

Commercial varieties of maize inbred for only one generation served as foundation stock for the crosses. To have inbred them more would undoubtedly have introduced another variable, namely, that the inbred line chosen might not have represented the main varietal characteristics. It would, however, have given a sharper definition to the resulting data. The following varieties were used:

[1] Paper No. 27. Department of Genetics, Iowa State College.

Golden Glow (GG)—16-rowed yellow dent, red cob.
Iodent (Iod)—16–18-rowed yellow dent, red cob.
Silver King (SK)—16-rowed white dent, white cob.
Evergreen (Ever)—16-rowed white sweet, white cob.
Crosby (Cros)—12-rowed white sweet, white cob.
Golden Bantam (GB)—8-rowed yellow sweet, white cob.
Black Mexican (BM)—8-rowed purple sweet, white cob.
Yellow Flint (YF)—8-rowed yellow flint, white cob.
White Flint (WF)—8-rowed white flint, white cob.
King Phillip (KP)—8-rowed red flint, white cob.

These varieties were crossed in many combinations, and F_2 and backcross generations grown. Only typical F_1 plants, distinctly intermediate in row number, were used to continue the crosses. The backcross progenies proved far more satisfactory than the F_2 because the inbreeding necessary to produce the latter not only gave weaker plants, but affected the straightness of the rows in many cases. For this same reason, F_3 progenies were very unsatisfactory.

In the subsequent tables I have presented only a small portion of the data. It should be clearly understood that these data are a picked lot, but included in the entire experiment are results both more and less extreme in nature. The full data in no wise point to any conclusions other than those given here. It is true that a few crosses do not exhibit the linkages presented herein, and in that sense the data do not present the whole truth. The details of the investigation, comprising at least 29,000 individuals, must be presented in another report.

In the following tables the probability that two comparative series of observations are independent is calculated by the chi-square test. The means of the two distributions are also included. Due to the nature of the material these two mathematical criteria only serve as indicators of the relationship—neither should be taken as conclusive in itself. The percentages (expressed as differences between the dominant and recessive characters) in the tables often give the best picture of the situation, because they indicate the trend of the varia-

tion, a very important consideration in this type of quantitative inheritance.

Number of Rows and Cob Color

Since no red-cobbed, eight-rowed varieties were known, the test for any linkage was restricted to the parental combinations of red cob-high row number and white cob-low row number. At least twelve such crosses were made. The data from a typical one are arranged in Table I.

TABLE I

Relation between cob color and row number in hybrid progenies
P_1–16-Iodent × 8-Golden Bantam

Rows	F_2 generation			12 F_1 × 8 GB			12 F_1 × 8 WF		
	*R**	*r*	Per cent. D**	*R*	*r*	Per cent. D	*R*	*r*	Per cent. D
8				24	62	−12.6	115	128	−4.0
10	3	4	−10.5	94	109	− 2.0	167	162	1.2
12	40	17	−19.6	116	101	9.5	64	54	2.8
14	46	6	22.6	19	10	3.8	2	2	0.0
16	17	3	5.7	3	0	1.2			
18	2	0	1.9						
Total	108	30	30.1	256	282	14.6	348	346	4.0
Mean	13.5	12.5		11.1	10.4		9.7	9.6	
P	0.03			0.0001			0.64		

* This symbol used to designate red cob (*R*) dominant to white cob (*r*), the complete formula being P^{er} and *p*, respectively.

** This column shows the percentage difference between the two distributions, the minus sign being used for the recessive gene in this and all following tables.

In all three hybrid generations there is consistent evidence that an association exists between row number and cob color in such a manner that the white-cobbed segregates possess a lower row number than the red-cobbed plants. The results are statistically significant in the F_2 and in the backcross of the F_1 to the parental eight-rowed Golden Bantam type. The consistency of the trends, however, would seem to be the best evidence

that a correlation exists. It is to be noted that it is the parental combinations that tend to appear with greater than normal expectancy. This is extremely suggestive of genetic linkage.

Whereas no eight-rowed, red-cobbed varieties are known, it is true that a red-pericarp, eight-rowed variety of maize exists. This is the King Phillip flint. Since pericarp color belongs in the same allelomorphic series as cob color, it should afford the basis for the necessary reciprocal test of any linkage situation. Accordingly I used this variety in crosses with colorless-pericarp varieties having the higher row number. But the pericarp color classification proved so variable that these crosses were quite unsatisfactory. It was perfectly evident, however, that there was a strong tendency for the eight-rowed and ten-rowed segregates in these crosses to carry the deeper red pericarp color, and a greater percentage of the ears with the lower row numbers were red. Cob color in these crosses was nevertheless distinct, and the results of one such cross are presented in Table II.

TABLE II

RELATION BETWEEN COB COLOR AND ROW NUMBER IN HYBRID PROGENIES P_1-8-King Phillip × 16-Golden Glow

Rows	F_2 generation			12 F_1 × 8 WF			12 F_1 × 8 YF		
	R	*r*	Per cent. D	*R*	*r*	Per cent. D	*R*	*r*	Per cent. D
8	14	9	−13.1	82	113	−13.6	23	48	−18.6
10	32	15	−10.8	50	45	5.3	41	49	1.5
12	30	5	21.3	26	16	7.1	22	9	17.1
14	2	0	2.6	3	1	1.3			
Total	78	29	23.9	161	175	13.6	86	106	18.6
Mean	10.5	9.7		9.4	8.9		10.0	9.3	
P	0.05			0.04			0.001		

Here the evidence of an association between cob color and row number is exceptionally striking. All three distributions consistently show that the plants with the lower

row numbers are inclined to possess white cob color. The probability values are all highly significant in demonstrating that the cob-color distributions are independent of one another; and the mean row numbers all reveal the fact that the white-cobbed plants possess a lower average row number.

There is, accordingly, in these two crosses an indication that row number is, to some extent at least, determined by something, very likely a gene, residing in the sixth chromosome, since this is the one bearing the cob (and pericarp) genes.

I have observed this same linkage of cob color and row number in many other crosses involving large populations and grown in different seasons. The same has been true of pericarp color, notably in crosses of Cardinal King (red dent, sixteen rows) by Golden Bantam. From such evidence I have become reasonably convinced of the existence of a generally wide-spread gene for row number localized on the same chromosome bearing the pericarp and cob color factors.

Number of Rows and Endosperm Color

Yellow and white endosperm colors in some varietal crosses are controlled by a single pair of genes *Yy*, borne on the fifth chromosome. In Table III such a cross is shown, in which the yellow color and high row number was one parental combination (Iodent-sixteen-rowed), and white color and low row number (eight-row White Flint), the other. In the F_2 generation there is a slight indication of linkage, which is more evident, however, in the backcross progenies. White endosperm color tends to be associated with the lower row number in all the hybrid progenies.

The reverse situation is exhibited in Table IV. Here are two backcross generations (the F_2 endosperm colors were not sharply enough differentiated) tracing to

TABLE III

RELATION BETWEEN ENDOSPERM COLOR AND ROW NUMBER IN HYBRID PROGENIES
P_1–8-White Flint × 16-Iodent

Rows	F_2 generation			F_1 × 8 WF			F_1 × Evergreen		
	Y	*y*	Per cent. D	*Y*	*y*	Per cent. D	*Y*	*y*	Per cent. D
8	0	1	−0.9	43	68	−7.7	0	2	−1.4
10	30	15	−2.2	100	93	2.2	6	11	−3.5
12	139	59	−0.4	163	151	3.9	54	57	−2.8
14	59	20	4.6	13	8	1.6	66	59	3.9
16	20	10	−1.5				17	15	1.2
18	1	0	0.4				5	1	2.7
Total	**249**	**105**	**5.0**	**319**	**320**	**7.7**	**148**	**145**	**7.7**
Mean	**12.6**	**12.5**		**10.9**	**10.6**		**13.4**	**13.1**	
P	**0.59**			0.06			**0.24**		

TABLE IV

RELATION BETWEEN ENDOSPERM COLOR AND ROW NUMBER IN TWO BACKCROSS PROGENIES

Rows	10-F_1(12-Crosby × 8-YF) × 8-WF			12-F_1(18-White Dent × 8-GB) × 8-WF		
	Y	*y*	Per cent. D	*Y*	*y*	Per cent. D
8	255	239	6.7	57	54	7.5
10	94	112	−3.8	46	57	−1.4
12	22	34	−2.9	46	66	−6.4
14	.....	.....	.....	2	2	0.2
Total	**371**	**385**	**6.7**	**151**	**179**	**7.8**
Mean	**8.7**	**8.9**		**10.0**	**10.2**	
P	**0.11**			**0.49**		

crosses in which the low row number was introduced by the yellow-colored parent. In both cases, the yellow endosperm color shows a tendency to be associated with the lower row numbers. Thus we have evidence that the tendency for row number and endosperm color to be correlated presumably traces to a genetic linkage and not to any physiological correlation. The intensity of this linkage appears not to be very marked. However, the uniformity of the results in the two reciprocal situations

leads me to suspect that the fifth maize chromosome bears at least a minor genetic factor determining row number.

Number of Rows and Aleurone Color

Aleurone color was introduced into the linkage tests by the eight-rowed Black Mexican sweet corn. A cross of Black Mexican by Golden Glow yellow dent is arranged in Table V. The F_2 data with their 9:7 aleurone ratio caused by the two factor pairs *RrCc*, exhibit no significant correlation between aleurone color and row number. The three backcross generations, on the contrary, do show a significant association, the purple color being appreciably correlated with the lower row numbers. The backcross progeny of 582 individuals involving the White Flint variety (*rrcc*) gives striking proof that aleurone color and row number factors are associated, but does not indicate which aleurone factor is involved.

Attention is called to the last backcross progeny of 759 individuals, that in which the F_1 was crossed with Yellow Flint (*rrCC*). Here a 1:1 aleurone ratio with the characteristic mottling of aleurone points to the fact that only the *Rr* aleurone genes are involved. There is good evidence here that these aleurone genes exhibit correlation with row number in a manner that suggests genetic linkage. Accordingly it would seem that the second chromosome of maize carries a factor for determining row number, since there is no reason to suspect that the linkage is due to any but a genetic cause. The linkage, if existent, is presumably very loose, however.

No crosses were made in which the higher row number was contributed by the parent possessing aleurone color. Hence the linkage relations of row-number factors and aleurone factors are not well established by any means. The results in other crosses have given variable relations, so that the linkage in question is the least convincing of any with which I have dealt. Knowing, as we do, that

TABLE V

RELATION BETWEEN ALEURONE COLOR AND ROW NUMBER IN HYBRID PROGENIES P_1–16-Golden Glow × 8-Black Mexican

Rows	F_2 generation			12-F_1 × 8-GB**			12-F_1 × 8-WF**			F_1 × 8-YF		
	*P**	*p*	Per cent. D	*P*	*p*	Per cent. D	*P*	*p*	Per cent. D	*P*	*p*	Per cent. D
8	7	13	−3.2	123	74	13.0	78	87	7.8	147	116	3.9
10	90	63	1.2	125	126	− 8.0	125	173	3.0	179	150	2.3
12	160	114	1.3	33	40	− 4.9	33	86	−11.0	71	80	−4.9
14	63	47	−0.3							6	10	−1.3
16	9	5	0.7									
18	2	1	0.2									
Total	**331**	**243**	**3.5**	**281**	**240**	**12.9**	**236**	**346**	**10.8**	**403**	**356**	**6.2**
Mean	**11.9**	**11.8**		**9.4**	**9.7**		**9.6**	**10.0**		**9.7**	**9.9**	
P	**0.46**			**0.01**			**0.003**			**0.17**		

* General symbol for purple aleurone color, comprising both *R* and *C* factors (9 : 7 ratio).

** The aleurone ratio on the ear was not maintained at planting time.

the second chromosome in maize undergoes much crossing over, it is perhaps not surprising that such a linkage would be difficult to detect under average circumstances.

Number of Rows and Starchy-sugary Endosperm

The starchy-sugary genes, *Su su*, belong in the third chromosome. They have been tested with row number in two sets of crosses: one in which the sugary gene was

TABLE VI

Relation between endosperm texture (starchy-sugary) and row number

P_1–16-Evergreen × 8-Yellow Flint

Rows	F_2 generation			F_1 × 8-GB			10-F_1 × 12-Crosby		
	Su	*su*	Per cent. D	*Su*	*su*	Per cent. D	*Su*	*su*	Per cent. D
8	19	0	8.1	129	73	17.9	19	13	6.6
10	69	15	2.6	58	60	−10.4	48	42	7.8
12	139	39	−10.4	4	13	− 6.7	31	44	−11.5
14	7	2	− 0.5	0	1	− 0.7	0	3	− 2.9
16	1	0	0.4						
Total	235	56	10.9	191	147	17.8	98	102	14.4
Mean	11.2	11.5		8.7	9.2		10.2	10.7	
P	0.16			0.001			0.09		

introduced into the cross with high row number (Table VI), and one in which sugary was brought in with the lower row number (Table VII).

The data in Table VI from the Evergreen x Yellow Flint cross show conclusively in all three generations (F_2 and two backcrosses) that, in this cross, there is a distinct tendency for the higher row numbers to be linked with the sugary character. Quite the reverse is true in Table VII (Golden Bantam x Golden Glow) where the sugary ears carry a higher percentage of the low row numbers.

Thus we have another case of a genetic linkage, which involves the third chromosome of maize. The intensity of the linkage in these particular crosses is reasonably

TABLE VII

RELATION BETWEEN ENDOSPERM TEXTURE (STARCHY-SUGARY) AND ROW NUMBER

P_1–8-Golden Bantam × 16-Golden Glow

Rows	F_2 generation			12-F_1 × 8-GB			F_1 × 12-Crosby		
	Su	*su*	Per cent. D	*Su*	*su*	Per cent. D	*Su*	*su*	Per cent. D
8	14	17	−16.5	41	45	−12.6	4	7	−1.8
10	36	21	− 1.1	71	48	4.1	36	44	−3.3
12	39	15	12.9	43	22	8.6	77	79	3.6
14	8	2	4.7				10	7	2.8
16							3	5	−1.2
Total	97	55	17.6	155	115	12.6	130	142	6.3
Mean	10.8	10.1		10.0	9.6		11.5	11.4	
P	0.06			0.06			0.71		

high as may be seen from the percentage-difference columns in both tables.

An exception to the above-described situation should be noted in the case of the Black Mexican Sweet x Golden Glow dent cross. Throughout this cross there was a slight tendency for the sugary segregates in the various hybrid progenies to be associated with the higher row numbers. Statistically little or no reliability can be placed on this correlation of the sugary character with higher row number since the differences were relatively small. The case is mentioned only to include the most extreme variation noted in a great amount of material that can not be recorded here. In general it must be added that linkages involving the sugary and row-number genes were more elusive than those concerned with cob color and row number.

CONCLUSIONS

Number of rows in the maize ear, a typical quantitative character, is shown to be associated in inheritance with such simple qualitative characters as cob, aleurone and endosperm color as well as endosperm texture

(sugary). The experimental evidence for genetic linkage between some of the multiple genes for row number and the genes for cob (and pericarp) color, endosperm color (*Yy*) and endosperm texture (*Su su*) is particularly convincing in certain crosses. That for aleurone color, especially the *Rr* genes, is extremely suggestive of a linkage. Accordingly it is highly probable that genes for row number are localized on the third (sugary), fifth (*Yy* endosperm color) and sixth (cob and pericarp) chromosomes in maize; and very likely on the second chromosome (*R*-aleurone) as well.

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EXPERIMENTAL EVIDENCE OF THE FUNCTION OF THE FIBRILLAR SYSTEM IN CERTAIN PROTOZOA

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In a memoir published in 1838 on organization in the Infusoria, Dujardin[1] concluded about as follows: Organs of the Infusoria possess a degree of organization that is in keeping with their manner of life and these organs are not comparable with the organ systems of higher animals. This statement was a refutation directed against claims advanced by Ehrenberg which in effect were that in these miniature animals, the Infusoria, it was possible to identify complex organs and organ systems that performed all the principal functions of animal life. Have Infusoria vision? According to Ehrenberg the conspicuous red eye-spot of Euglena represented a highly differentiated visual organ. As regards a digestive system, he described and illustrated for *Enchelis, Leucophrys* and other ciliates a permanent and complete alimentary tract having a mouth and anal pore. Other organ systems were also in evidence: an elaborate reproductive system provided with a testis (a structure we now call the nucleus), a seminal vesicle (the contractile vacuole), and numerous eggs (the granules distributed throughout the body). In addition, Ehrenberg urged, although he did not identify, for these microscopic animals a complex nervous, muscular and circulatory system.

These premature interpretations were early called in question and were successfully refuted during his own lifetime. His first and probably foremost critic was Dujardin, who is now remembered rather as the discoverer of the living substance composing these lower forms. This historic discovery of sarcode, it is interest-

[1] *Ann. Sci. Nat.*, 1838, p. 312.

ing to note, was made and established during this controversy on the nature of organization in the Infusoria.

Dujardin confined his investigations chiefly to the root-forming organisms to which he gave the name Rhizopoda. In these he observed that highly complex organs were conspicuously wanting. Also, in various Infusoria, by extensive feeding experiments, he demonstrated that a complete alimentary tract did not exist, but that digestion was performed in impermanent and individual food vacuoles such as we know them to-day. He further showed that the contractile vesicle, supposedly part of a reproductive system, was a fluid, transient vacuole which afforded a continuous intake and expulsion of water, probably with a respiratory function. Finally, Dujardin asserted that he was unable to find any trace of a nervous, muscular or circulatory system.

These and later negations of Ehrenberg's claims helped especially toward clearing the way for a more acceptable theory of organization in the Infusoria. It remained for von Siebold to propose that theory, *viz.*, that Infusoria are unicellular organisms. Announced in 1845, a favorable time, the theory rapidly gained many adherents. Its chief virtue proved to lie in interpreting the endoplast, or central body of the Infusoria, as homologous with the ordinary nucleus of tissue cells.

On the basis of a simple, unicellular structure most of the diversified group of Infusoria were now assembled by von Siebold under the title of the Protozoa. Dujardin's contentions against a highly complex organization in members of this group were more fully recognized, and soon the pendulum's swing tended toward extreme interpretations of simplicity of structure. Cellularity, as commonly applied to tissue cells, became the salient feature of all Protozoa. They came to be generally regarded as the most primitive and simplest forms of animal life.

With some such historical background we may perhaps better approach the subject proposed for our consideration.

During the three quarters of a century since these engaging questions of organization in the Protozoa were vigorously debated, a vast body of literature has appeared. Much of it has had to do with similar questions of organization. With the aid of improved technique we have been given a clearer picture of the diverse structures of many Protozoa and we now know something more about the functions of these structures. We have come to see, too, that the results of researches in more recent years, especially for the ciliates, point toward complexity, rather than simplicity, in their organization.

Among the diverse kinds of structures that later investigations have disclosed, both for flagellates and for ciliates, we are concerned to-day particularly with an integrated system of fibrils which are intimately associated with organs of food-taking and locomotion in the ciliates.

In 1914, Robert G. Sharp, working under the direction of Professor Kofoid, at the University of California, published the results of his studies on a ciliated protozoan, *Diplodinium,* which is parasitic in the stomach of the ox. In this ciliate Sharp found a remarkable system of fibrils which were associated with the different motor organelles of the oral region. Owing to the shape, position, relations and staining properties of this fibrillar system, he regarded it as having an unusual significance.

Inasmuch as this complex fibrillar system is intimately associated with the motor organelles, Sharp was justified in regarding these fibrils as part of the animal's motor mechanism, whatever their specific rôle might be. But just what is their specific function? Three possibilities were obvious: (1) this fibrillar system may be skeletal for support; (2) it may be muscular, the strands representing primitive contractile fibers, or (3) these strands may have conductive properties with the motorium functioning as a sort of coordinating center for impulses over the fibrils to the various motor organelles. After weighing the evidence which his investigation had disclosed,

Sharp concluded that the last hypothesis was in nearest agreement with the facts. Accordingly, he gave to this system the name "neuromotor apparatus."

In 1918, H. B. Yocom, working also in Kofoid's laboratory, found and described in the fresh-water ciliate, *Euplotes patella,* a fibrillar system comparable with that of *Diplodinium ecaudatum.* Griffin[2] (1910) had observed in part a similar fibrillar complex in *E. worcesteri.*

Yocom's morphological studies were made chiefly on fixed and stained material. Following his figures and description, I later succeeded in identifying in the living, unstained organism, or during its gradual disintegration, all the chief elements of the fibrillar system noted by Yocom. In this manner I was further able to offer certain minor modifications and additions to his account.

In order better to understand the various connections of the fibrillar apparatus in *Euplotes patella,* it will be advantageous to offer briefly a description of this organism, especially of the relative position and structure of its external, motor organelles.

The body of Euplotes (Fig. 1) in general contour roughly resembles an inverted bowl of a tablespoon, the convex surface of which represents the dorsal side of the animal and the concave surface its ventral side. For the *anterior end,* to complete the figure, one should picture a mere stub of a broad handle still attached with its free edge well rounded to suit the general contour of the bowl. This stub would then represent the animal's oral lip. Essentially, this lip forms an anterior projection of the dorsum over a wide triangular cytostome at the posterior apex of which is the pharynx situated about half way down the body.

A series of membranelles, located on the dorsal side of the oral lip, extends anteriorly from its base. On the left, these membranelles continue and turn ventrad to border entirely the left side of the cytostome and pharynx.

[2] *Phil. Jour. Science,* 5: 291–314.

The remaining external organs embrace eighteen pencil-shaped cirri. Of these, four are caudal and fourteen ventral in position. The largest and longest of those on the ventral side are a group of five anal cirri, situated about twenty-five microns from the posterior end, and commonly extending backward and beyond the caudal margin of the body.

The ciliary composition of both cirri and membranelles

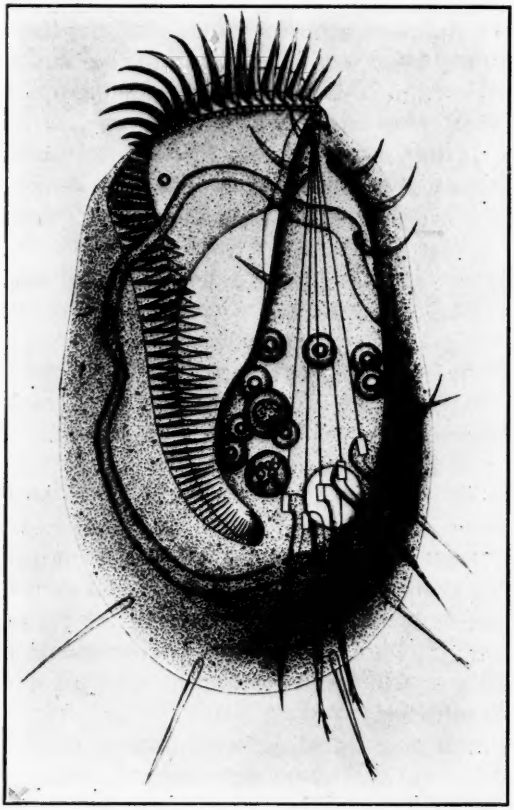

FIG. 1. Semi-diagrammatic dorsal view of *Euplotes patella* illustrating its fibrillar system and the relation of that system to the locomotor organelles.

occurring in the ciliates is a well-established fact. This may be readily demonstrated in a shallow hanging drop by means of the micro-needle. Here, for example, a detached cirrus may be pushed near to the edge of the drop and rolled to and fro between needle and cover-slip. Soon the cirrus splits into loose bundles of its component cilia. Some minutes after, upon further disintegration, one can clearly identify the differentiated parts of each cilium, *viz.*, the axial filament, the basal granule and the ciliary rootlet. It is evident, too, that these are embodied in a gelatinous matrix which appears to be highly viscous and glutinous. It is important to note that the basal granule and ciliary rootlet are enclosed within a firm and fairly well-defined basal plate. This is true for each cirrus and each membranelle.

Intimately associated with these locomotor organs, the anal cirri and membranelles, is a unified fibrillar complex.

For our consideration, it is especially important to observe that the basal plate of each cirrus and of each membranelle is affixed to, but not continuous with, a conspicuously differentiated, relatively thin, platelet. These may conveniently be designated respectively as (1) the anal fiber plate, and (2) the membranelle plate. Continuous with the fiber plate of each cirrus is an elongated fibril that extends anteriorly toward the extreme right corner of the body. Here each of these five anal cirri fibers converge and unite to one end of a small but fairly distinct bilobed mass, "the motorium." From the opposite end of this motorium passes another fiber to which are connected the membranelle plates located at the bases of the series of membranelles. The membranelle plates are entirely comparable with the anal fiber plates.

It is obvious that this system of fibers affords morphological continuity between the five anal cirri and the entire series of membranelles.

It should be noted also, that at the base of each of the other fourteen cirri is a fiber plate from which extend several (three to five) short fibers whose free ends fade

out in the surrounding ectoplasm. Apparently these "dissociated fibers" are not morphologically continuous with the unified system of fibers described above.[3]

EXPERIMENTAL

The morphological evidences which these and other investigations have yielded establish beyond doubt that for these ciliates their fibrillar mechanism is unified and is intimately associated with their external organs of locomotion and food-taking. These facts alone lend support to the claim that such a unified system of fibrils performs a function more highly specialized than merely that of support, or even one of contractility. Yet, however significant may be the morphological evidences favoring the function of conductivity or any other function for such a system, of course methods beyond the bounds of morphological inquiry must be introduced.

During the winter of 1916–17 when Dr. Yocom had found and was studying the fibrillar system in *Euplotes patella,* it seemed to me that the experimental method of microdissection might be successfully employed to aid in determining the actual function of this internal mechanism in *Euplotes* and so supplement Yocom's excellent morphological studies by experimental evidence.

The microdissection method, introduced some twenty years ago by Dr. M. A. Barber, has since been so extensively employed that it is doubtless more or less familiar to all who are present here to-day. In due tribute to Dr. Barber, I take this opportunity to express in some measure an appreciation of the method he instituted and the simple and efficient device which he originated for the accurate control of micro-instruments under the high powers of the microscope. It was by means of one of his manipulators and a moist chamber of his design that these experiments on *Euplotes* were carried out more than a decade ago. Barber's method, which has now been

[3] For an account of similar fibrillar systems in other ciliates, the reader is referred to an article by Professor Calkins.

variously modified and improved by Chambers, Peterfi and others, affords for the hand a precise and reliable aid comparable with that which the microscope provides for the eye, and for a wide variety of researches on the living cell we have as yet not fully realized the possibilities of that method.

In order to control for dissection experiments this exceedingly active ciliate, whose name *Euplotes,* meaning good swimmer, is indeed well-chosen, the surface film method similar to that described by Kite (1913)[4] was found adequate. This means of control is particularly efficient for a micro-organism such as *Euplotes,* whose rigid pellicle maintains the normal form of the body, even after a fairly deep incision has been made. The method, furthermore, permits the organism to be readily oriented by means of the dissection needle in order that it may be cut in any desired plane.

The various cuts made in these experiments included (1) transections, severing the organism into two parts, (2) excisions of external organelles, with or without a portion of the body, and (3) incisions into the body or oral lip in any suitable plane.

It is neither possible nor necessary in this brief report to state more than the general results that followed these three types of dissections. The several kinds of transections made on *Euplotes patella* revealed one distinct difference in the behavior of the resulting pieces which is important for our consideration. The normal locomotion of this ciliate includes three creeping movements: (1) straight ahead, (2) a quick backward movement and (3) a sharp turn to the right (aboral); also, for our present purposes, three swimming movements: (1) forward in spiral revolutions, (2) circus movements to the right (aborally) and (3) swimming rapidly backwards.

Following a transection made at right angles to the long axis about midway through the body, the anterior

[4] *Amer. J. Physiol.,* 32: 146–164.

piece tends to swim continuously in right circus movements while, on the contrary, the posterior piece revolves rapidly about its cut surface as an axis. This marked difference in the behavior of the anterior and posterior pieces well illustrates the significant finding that in *E. patella* one of the several common movements of locomotion prevails in a piece formed by transection. It shows, also, that the elimination of any important group of organelles, or the interference with any mechanism by which they operate or cooperate with another similarly important group tends to limit the kinds of movements which any such group may and does perform. These differentiated, diverse parts are, accordingly, complementary parts, so that the usual creeping and swimming movements of the organism as a whole depend indispensably upon the coordinated activity of all its diversified motor organelles.

This feature of specific behavior of isolated pieces of the organism was all the more evident in *excised* smaller pieces.. For example, upon removal of the adoral membranelles including the oral lip but devoid of other locomotor organelles, the behavior of the resulting excised piece was conspicuously stereotyped, constantly swimming in rapid circus movements until it disintegrated, perhaps some fifteen minutes later. Similarly, the excision of the caudal region of the body having only the caudal cirri resulted in the piece revolving very rapidly with its cut surface as an axis but in such manner as to move speedily through the water with the left side foremost.

Other excisions, the results of which are of special significance, include the removal of a single anal cirrus, or any other of the eighteen cirri, at its base, and the excision of as few as four membranelles. In each of these detached organelles I have distinctly observed contractions, which in some cases continued for several seconds. These results demonstrate conclusively that the contractions of anal, frontal or caudal cirri or of membranelles

of *Euplotes patella* are not conditional upon attachment to the body and, therefore, not upon any mechanism within the body.

Evidence which tended to reveal the actual function of the fibrillar mechanism of *Euplotes* was obtained upon making incisions variously in the animal's body. For present purposes, it will be sufficient to note briefly the results of four kinds of these incisions:

(1) Through the oral lip without cutting the membranelle fiber. The results were wholly negative in all of many cases.

(2) Through the oral lip, severing the membranelle fiber. In seventeen cases there resulted abnormal swimming movements and distinct changes in the movements of the membranelles on either side of the incision. Upon examining with high power the movements of the membranelles, a difference in rhythm was frequently conspicuous between the series on the left side of the cut and those on the right side. Also, though less frequently, the series on one side of the cut were distinctly seen to beat with the effective stroke in one direction, while the membranelles on the other side of the cut beat with the effective stroke in the opposite direction. The contrary movements of carmine granules or of particles of Chinese ink which had been introduced into the water indicated these striking differences in the opposite directions of the effective strokes. These results constituted the first definite evidence of induced interruption of coordinated activity in the locomotor organs of *Euplotes*.

(3) Cutting the anal cirri fibers at any point between these cirri and the motorium, or destroying the motorium. The general effects upon swimming or creeping movements and upon the normal coordination of the effective strokes of anal cirri and membranelles were definite, fairly constant and much the same after performing any of these incisions. There was distinctly less tendency to creep, but when creeping was attempted it was sometimes clearly evident that the frontal cirri initiated the move-

ment which was then taken up by the anal cirri. This succession, however, was not always so evident and in four cases it could not be observed. The normal avoiding reaction was seldom seen at any time after such an incision had been made. If the oral lip were touched by the needle (a stimulus which normally induces the avoiding reaction), the incised animal would infrequently exhibit this reaction, but more often would only turn to the right, thus avoiding the stimulus without performing the preliminary backward movement.

(4) Incisions on the right or left side or at the posterior end which did not sever the membranelle fiber or any of the anal cirri fibers. Following such incisions made in many *Euplotes* at various angles I at no time observed any noteworthy change in their normal swimming or creeping reactions or in the perfect coordination between the series of membranelles and the anal cirri.

Perhaps the clearest evidence for the want of coordination and of concomitancy of movements between the membranelles and anal cirri appeared in these incised animals upon supporting one of them against the under surface of the cover glass with a very flexible needle. To the hanging drop had been added a trace of Chinese ink or a carmine solution; thereby, changes in the direction of the effective stroke either of the anal cirri or of the membranelles were made evident in corresponding movements of the particles of ink or of carmine. At times the particles were driven in the same direction by the membranelles and by the anal cirri, hence the effective stroke of these organelles operated synchronously. This concomitancy, however, did not always continue. The direction of their effective strokes changed so that now while the membranelles were driving some particles anteriorly, other particles were being driven posteriorly by the anal cirri, or *vice versa*.

In the interpretation of the experimental evidences briefly reviewed in the foregoing paragraphs, two preliminary questions suggest themselves: Does the fibrillar

system in *Euplotes patella* represent a mechanism that affects the external organelles individually? Or does this complex, unified apparatus function in the coordination of all the several groups of organelles with which it is intimately associated? An affirmative reply to the first question would assign either a supporting or a contractile function to this system, and to affirm the second question is to attribute to the system the function of conductivity.

My experimental evidences support an affirmative answer to the second question, *viz.*, that this fibrillar system in *Euplotes* does function to coordinate the groups of external organelles with which it is intimately associated. These evidences, furthermore, appear not to support the assumption that the system is either contractile or supporting in function.

The rigid, fairly tough pellicle is amply sufficient to maintain the normal shape of the body under considerable stress, even after an incision through the anal cirri fibers about two thirds the width of the body has been made. Accordingly, the fibrils offer no discernible structural support to the animal's body.

It was noted that the basal granules and their ciliary rootlets, both for membranelles and cirri, are imbedded in a gelatinous ectoplasmic basal plate. This basal plate, in turn, is contiguous with, but not attached to, the fiber plate, as indicated by the readiness with which the basal plate becomes detached from the fiber plate, and by the want of any signs of attachment of the ciliary rootlets to the smooth fiber plate. It is apparent, then, that the basal plate which is continuous with the gelated, firmly supported ectoplasm, and not the fiber plate, is the means of secure attachment and support for both cirri and membranelles.

We have also noted that the contractility either of cirri or of membranelles is not conditioned upon their attachment to the body and consequently not upon any mechanism within the body. Moreover, the reversibly effective

strokes of the anal cirri preclude the possibility that the anal cirri fibers are contractile in function. Since contractile fibers can operate effectively only in one direction, it is not conceivable that an anal cirrus fiber can function as a contractile organelle. It is important and significant in this connection to recall that the severing of the anal cirri fibers did not incapacitate either the forward or the backward effective stroke of any of the anal cirri.

The remaining alternative, then, calls for evidences which demonstrate a conductive, coordinating function for this unified fibrillar mechanism in *Euplotes*.

We have seen that an incision at any point through the oral lip, which did not sever the membranelle fiber, gave negative results. But when the membranelle fiber was severed, there were changes in rhythmic movements of the membranelles on either side of the incision and modifications in the animal's swimming movements. Also, the effective stroke of membranelles on one side of the incision was observed to be at times opposite to that of the membranelles on the other side of the incision.

Destruction of the motorium or cutting its attached fibers interrupted coordination in the movements of membranelles and anal cirri. It will be recalled that here, as with the membranelles, the direction of the effective stroke of the organs on one side of the incision was occasionally observed to be directly opposite to that of the organs on the other side. These comparable results, perhaps more than any other, demonstrated the actual coordinating function of the fibrillar system; for any incisions through any region of the body which did not sever or injure the complex of fibers neither impaired the perfect coordination of the membranelles and anal cirri nor modified the animal's normal creeping and swimming movements. We may, therefore, regard these morphological relationships as conditioning the animal's usual behavior both in creeping and in swimming.

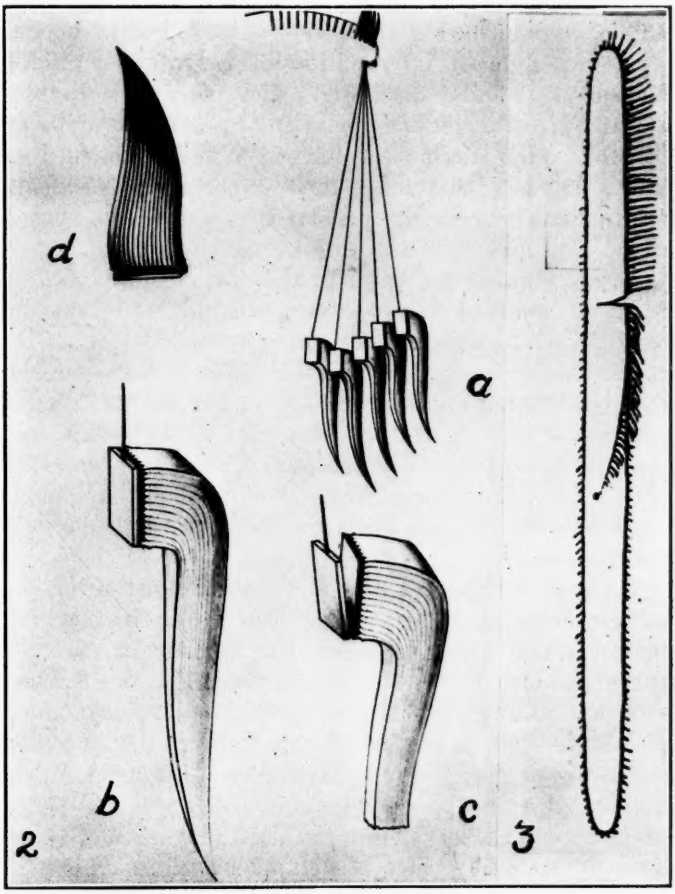

FIG. 2. Isolated parts of the fibrillar system and its associated locomotor organelles (diagrammatic). a. Portion of the system intact, showing anal cirri fibers and fiber plates, and the membranelle fiber and membranelle fiber plates. b. Anal cirrus with its fiber plate contiguous with its basal plate. c. Anal fiber plate separating from the basal plate of an anal cirrus. d. Membranelle and its fiber plate.

FIG. 3. *Spirostomum ambiguum.* Interrupted coordinated movements of the membranelles following an incision (after Verworn).

It should here be stated that these experiments on *Euplotes,* published in 1920, were not the first to demonstrate an induced interruption of coordinated ciliary movement. Similar dissection experiments were carried out on *Spirostomum ambiguum* and *Stentor coerulens* by Verworn with similar results which were published in 1889.[5] His brief account of these important experiments was unknown to me until about four years ago when I chanced to find it. Upon making incisions by means of a scalpel between the bases of the oral membranelles of *Spirostomum* and *Stentor,* Verworn observed that the behavior of the membranelles on opposite sides of the incision was different (Fig. 3). He states: "It could be plainly seen that the wave movement of the membranelles progressed only up to the incision, but not beyond it, and that the mean position of the membranelles on one side of the cut was different from that on the other side." These results are in accord with those which I obtained for *Euplotes.*

Verworn attributed this interrupted behavior of the membranelles in his experiments to his having severed the ectoplasm which he believed to transmit the coordinating impulses. It is of interest to recall in this connection the findings of Maier (1903) on the structural differentiations in the motor apparatus of both *Stentor* and *Spirostomum.* For each of these organisms Maier discovered a fibrillar complex associated with the bases of the membranelles. From the basal plate of each membranelle he could trace a well-defined fibril which, in *Stentor* (Fig. 4), clearly joined a longitudinal thread that thus united the entire series of membranelles. If we should attribute a conductive function to this fibrillar system, it is quite possible that Verworn actually severed the longitudinal thread in his experiments, thereby interrupting the coordinated movements of the motor organelles.

[5] "Protisten-Studien," Jena, 1889.

These important findings of a differentiated unified system of fibrils in widely separate groups of Protozoa lead one to suspect that other comparable systems will be found to occur, perhaps generally, throughout all the groups having well-defined locomotor organelles. Such prospects seem promising and offer an extensive field of investigation for those interested in the finer morphology

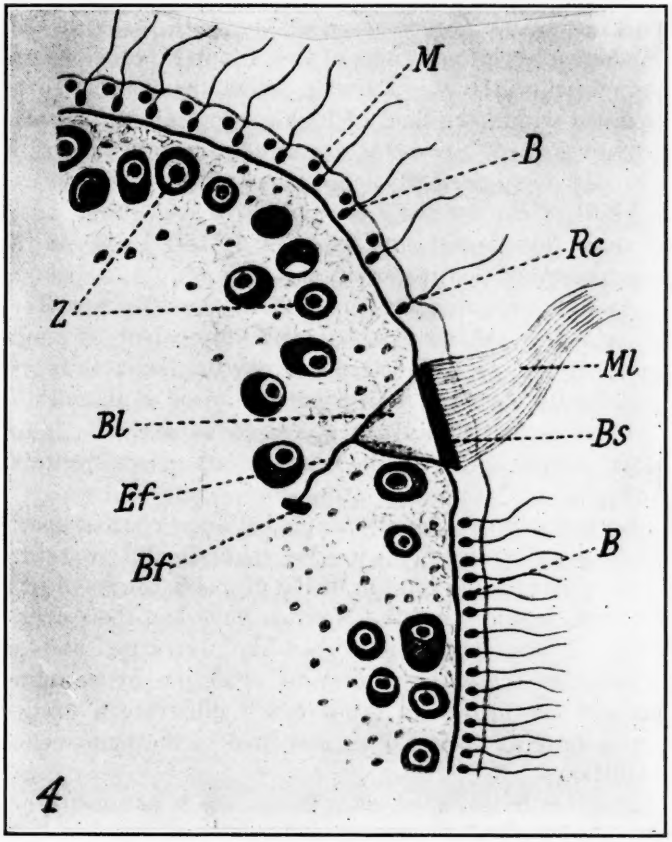

FIG. 4. *Stentor coerulens.* Cross-section showing a membranelle from the base of which extends a fiber that joins a larger longitudinal fiber which in turn thus unites the entire series of membranelles.

of these miniature forms of life. Just what the actual function or functions of such systems may be remains to be determined by precise methods of experimentation, as far as possible for each species. While only a beginning in this direction has thus far been made, it is clear that modern methods of microtechnique now make possible the solution of many of these experimental problems.

The importance of their solution none would question. Such problems are basic. They have to do with that fundamental characteristic of living matter which we call organization. Here in a living, active, transparent form, confined within the field of high magnification, one may see diversified, protoplasmic structures, all integrally associated and perfectly coordinated. Such a picture can not fail to clarify one's concept of organization. And through this clearer concept one may better discern the true nature of the protozoan organism.

As first proposed by von Siebold, the Protozoa were conceived to be the morphological equivalent of single cells. By virtue of this unicellularity they came to represent the simplest and most primitive forms of animal life. Cellularity, accordingly, served as the essential basis for their comparison with the great group of multicellular animals.

Such a comparison is useful and in certain respects altogether justifiable. It is a logical and valid procedure in formulating our concept of the probable course of evolution to assume that the Metazoa have had their origin from a unicellular, protozoon-like ancestry, and to attempt to trace this course of evolution by selecting present-day protozoan types which illustrate a graded series ranging from the single-celled to the many-celled condition.

But if it be assumed, as it commonly is assumed, that the Metazoa and Protozoa have had a common origin, then the latter must be equally as old as the former, and whatever primary factors have been involved in the evo-

lution of the Metazoa have similarly played a rôle in the evolution of the Protozoa. We should expect, therefore, to find evidences of evolutionary changes in the Protozoa of the present time no less certain and perhaps little less remarkable than those that obtain in present-day Metazoa. The marvelous diversity in the form and structure of many protozoan organisms, their universal occurrence in every known type of environment and their capacity for adaptation against the exigencies of nature—all these give evidence of the changes wrought through evolution. All in all, the Protozoa are apparently as well qualified to fulfil the requirements for livelihood and for the propagation of their kind as are the Metazoa. They, too, have learned how to get on in the world.

In these respects, at least, we are to regard them as something more than simple, primitive cells, and surely something more than the morphological equivalent of a single tissue cell. So obvious seems to be this truism that it might suitably invite the question: when is a cell not a cell? and the reply: when it is a unicellular organism. For in a very real sense the Protozoa are organisms. The degree of differentiation of their diverse and integrated structures that exhibit perfect, *yet modifiable,* coordination, such as these investigations seem to have demonstrated, should give them due rank in this category of individual organic forms, which bear the marks of a simple cellular origin.

THE NATURAL HISTORY OF CLADOCERANS IN RELATION TO TEMPERATURE

II. TEMPERATURE COEFFICIENTS FOR DEVELOPMENT

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I

IN an earlier paper[1] (Brown, 1929) it was shown that there is a close correlation between the geographical range and seasonal distribution of different cladoceran species and the temperature limits, both upper and lower, for their vital activity. It was found that the common species could be divided into three groups according to their distribution, namely, widespread, southern and northern; and, further, that the widespread species were divisible into those having maximum numbers of individuals in the summer and those having maxima in both spring and fall. The present paper reports a series of experiments to determine the relation of the rates of development at each of several temperatures of certain of these species to their known distribution.

If the velocity of a reaction at $t°$ C. is obtained and a second velocity at $t-10°$ C., the quotient between these two velocities is called the temperature coefficient or the Q_{10}. The Q_{10} for temperatures less than ten degrees apart may be obtained by the equation,

$$Q_{10}=\left(\frac{k_1}{k_0}\right)^{\frac{10}{t_1-t_0}}$$

where k_1 and k_0 are the rates or velocities at the temperatures t_1 and t_0. Instead of the actual velocities, quantities directly proportional to them serve equally well.

Much work has been done in applying the temperature coefficient to biological phenomena. Kanitz (1915) and

[1] Work done while at the Zoological Laboratory, Harvard University.

Przibram (1923) have gathered these data together and have attempted to analyze them. A reader is struck by the vast assortment of vital activities which show somewhat similar temperature coefficients. But since in many cases the data existing in the literature are recognizably deficient because the organism used lacked genetic uniformity or lacked the prerequisite uniform laboratory cultural conditions, the cladocerans were considered as admirable material for this work as they fulfilled both requirements.

II

The six species of Cladocera used and their geographical range and seasonal rhythm are as follows (Brown, 1929); *Moina macrocopa,* widespread with summer maximum; *Pseudosida bidentata,* southern; *Simocephalus,* widespread, with spring and fall maxima; *Daphnia pulex,* widespread with spring and fall maxima; *D. longispina,* widespread with spring and fall and occasionally winter maxima; *D. magna,* a northern form with the seasonal rhythm unknown. One of the species, *D. pulex,* included two varieties, one being the typical form and the other the "984" clone (Banta, 1925; Schrader, 1925) which was known to be different from the type form. The animals used throughout the experiments were all from one clone, that is, they were all descendants, by parthenogenesis, from one female. They were reared at laboratory temperatures in a suitable culture medium (Banta, 1921) until a large brood of females was obtained. These females, when about ready to release their first broods, were transferred to temperature-control cabinets. These cabinets were heated by carbon-filament bulbs and the temperature control was by means of a mercury thermoregulator. The temperature range for a given cabinet was $\pm 1.0°$ C. When the females had released their young, the time of release was recorded, and the young animals allowed to develop at that temperature. These animals passed through several moults and became adult. The end of the first adult instar is marked by the release

of the first brood of young. When the time of this release was noted and compared with the previous time recorded, a measure of the time for a complete parthenogenetic generation at a given temperature was obtained. The rate of development was then obtained by taking the reciprocal of the time required by the female in passing from the beginning of the first young instar to the end of the first adult instar.

The following table (Table I) gives the data so obtained. The species are arranged according to the relative magnitudes of the temperature coefficients for the temperature interval between 20° and 30° C. The lowest temperature used in these experiments was 13°, as below this temperature the time required by two species, *P. bidentata* and *M. macrocopa,* was so long in comparison to the time at higher temperatures for the completion of a generation that disturbing factors, indirectly due to the low temperature, were apparent. The latter species sinks to the bottom of the culture bottle at 10° and is ultimately killed by the accumulation of debris within the carapace.

TABLE I

LENGTHS OF THE GENERATION CYCLE, FROM BEGINNING OF THE FIRST YOUNG INSTAR TO THE END OF THE FIRST ADULT INSTAR, FOR SEVERAL COMMON SPECIES OF CLADOCERANS. IN THE FIRST VERTICAL COLUMN FOR EACH SPECIES ARE GIVEN THE TIMES IN HOURS FOR THE COMPLETION OF THE CYCLE; IN THE SECOND VERTICAL COLUMN ARE GIVEN THE VALUE OF Q_{10} FOR 13° TO 20°, 20° TO 30°, AND 25° TO 35°. THAT NOT ALL SPECIES WERE RAISED AT EACH TEMPERATURE IS SHOWN BY THE TABLE. THE SPECIES ARE ARRANGED ACCORDING TO THE RELATIVE MAGNITUDE OF THE TEMPERATURE COEFFICIENTS BETWEEN 20° AND 30° C.

° C	Moina macrocopa		Pseudosida bidentata		Simocephalus all species		Daphnia pulex (type)		Daphnia longispina		Daphnia magna		Daphnia pulex (984)	
	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}	time in hours	Q_{10}
13	296.8	3.96	517.6	2.90	381.0	2.36	309.7	2.64	284.5	1.82				
20	114.2		246.0		208.5		156.8		187.0		203.1		170.3	
25	66.9	2.39	134.3	2.03	129.1	1.81	100.8	1.70	138.0	1.48	146.0	1.31	120.4	1.19
30	49.5	1.76	121.2	1.63	115.0	1.15	92.1	•	126.0		155.0		143.2	
35	38.0		82.4		112.5		Will not live and reproduce							

III

All the species for which there are adequate data show a decrease in the value of the temperature coefficient with increasing temperature, the Q_{10} for the temperature interval 13° to 20° being greater than for any higher temperature interval. In general, the values of the temperature coefficients obtained with cladocerans are well within the range of values reported by Kanitz (1915) and by Przibram (1923) for rates of development of many animals and of arthropods in particular.

The rates of development for these species are plotted (Figure 1) against the Centigrade temperature. A few points of interest are apparent in this figure that might be overlooked in the preceding table. This figure shows, for example, that *M. macrocopa* is quite differently influenced by increasing temperatures than the other species. This figure also reveals the fact that two forms, *D. magna* and *D. pulex* "984," develop at a slower speed at 30° than they do at 25°. In other words, the "optimum" temperature for growth is evidently below 30° C.

The species of Cladocera studied may be divided into groups on the basis of their temperature coefficients for rate of development between 20° and 30°. The first group includes *M. macrocopa,* with Q_{10} equal to 2.39 and *P. bidentata* with Q_{10} equal to 2.03. The second group includes the species of *Simocephalus* with Q_{10} equal to 1.81 and the typical variety of *D. pulex* with Q_{10} equal to 1.70. *D. longispina* stands alone with 1.48 as the value of the temperature coefficient. The fourth group consists of *D. magna,* having a temperature coefficient equal to 1.31, and *D. pulex* "984" with Q_{10} equal to 1.19. It is at once apparent that these groupings correspond rather closely to the relative positions of the lethal temperatures of these same species (Brown, 1929) and to what may be termed their habitat groupings (geographical range and seasonal periods of abundance). It may also be pointed out that cladocerans, although reared for years side by

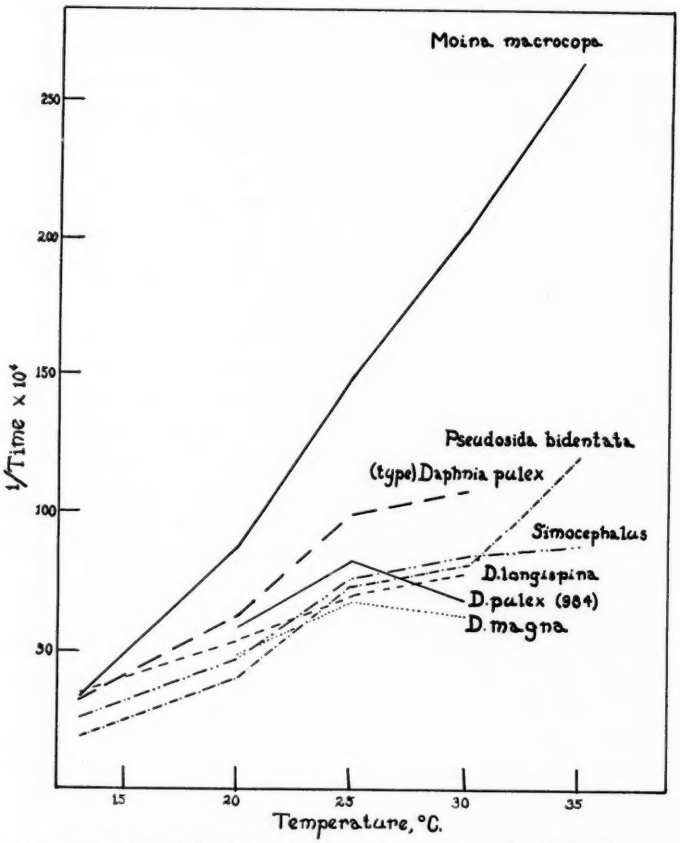

FIG. 1. Rates of development expressed as reciprocals of the time required to pass from the beginning of the first young instar to the end of the first adult instar, plotted against the Centigrade temperature. The rates for the development of the different species are nearly the same at 13° but widely different at 30° and 35°. Many of the forms are unable to live and reproduce at this latter temperature.

side in the laboratory under identical environmental conditions, retain specific differences when the temperature is varied. If acclimatization were a factor there would be a tendency for the values of the temperature coefficients for development to approach some average value for all the species so reared.

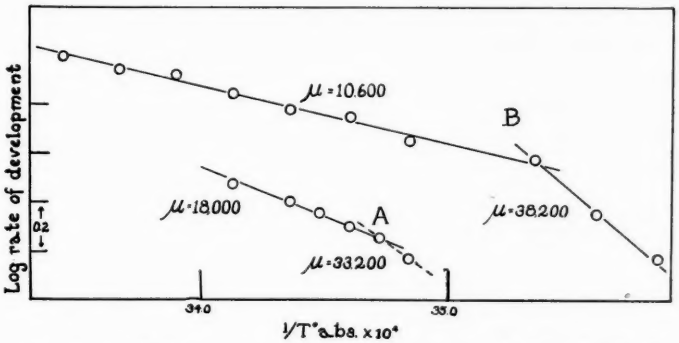

FIG. 2. *A. Dytiscus marginalis. B. Dytiscus semisulcatus.* Graphs for rates of larval development plotted against the reciprocals of the absolute temperatures. The values of μ are given opposite the segments of the graphs. The break in the graph for *D. semisulcatus* occurs at 10° C. A break possibly occurs in the graph for *D. marginalis* at 15° C. (data from Blunk, 1923).

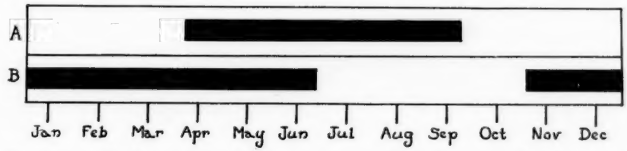

FIG. 3. *A. Dytiscus marginalis. B. D. semisulcatus.* Graphs showing the seasonal occurrence of the two species of beetle larvae for which the rates of development are given in Fig. 2 (data from Blunk, 1923).

A further check on the reality of the differences of cladoceran species in relation to temperature is given by a consideration of the temperature characteristics for the duration of a single adult instar (Brown, 1926–27). The values of the temperature characteristics and the position of the breaks in the *log rate* against *reciprocal absolute temperature* graphs were afforded a natural interpretation in terms of the distribution and seasonal rhythms of the three species studied. Data on the speed of larval development and seasonal occurrence of *Dytiscus* beetle larvae (Blunk, 1923) serve as another striking example of this relation (Figs. 2 and 3).

SUMMARY

Summer and southern species of cladocerans have higher temperature coefficients for development than do those of more general distribution. This was shown to be in conformity with the data for the temperature characteristics for the duration of a single adult instar and with the lethal temperatures for the same species.

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OBSERVATIONS ON THE ATTACHMENT OF *BALANUS CRENATUS* BRUGUIERE FOUND IN THE WATERS OF PUGET SOUND

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THE observations on the settlement of the cyprids of *Balanus crenatus* Bruguiere, a species of barnacle found on the Pacific Coast, afforded a very enjoyable part of a piece of research concerning the fouling of ship bottoms moored in Puget Sound. This work was done under the guidance of Professor John Weinzirl, of the bacteriology department of the University of Washington, Seattle.

HISTORY

Probably no one has devoted as much time to the classification of barnacles as Charles Darwin (4). In his priceless monograph of 1853[1] (5) (p. 261) he quotes from the Encyclopedia Methodique (4) of 1789 the wide distribution of *Balanus crenatus* Bruguiere. "Its habitat ranged from Great Britain, Scandinavia, Arctic regions, as far as Lancaster Sound in 70°–48′ North (Mr. Sutherland), Behring Straits (Capt. Kellett), United States, Mediterranean, West Indies (Mrs. Brit.), Cape of Good Hope (Mrs. Kraus). Generally it is attached to shells and crustacea in deep water, sometimes to ship bottoms. Very common." On page 264 he states that he had received specimens from all parts of the coast of Great Britain and Ireland, generally attached to Crustacea and Mollusca, and never hitherto from rocks uncovered from the tide. Some specimens were obtained from Pinna, from about fifty fathoms. Pilsbry's (11) (1916, p. 171) American records are from low tide to fifty fathoms,

[1] The edition in the University of Washington library is dated 1853, while Pilsbry and other workers report the same edition as 1854.

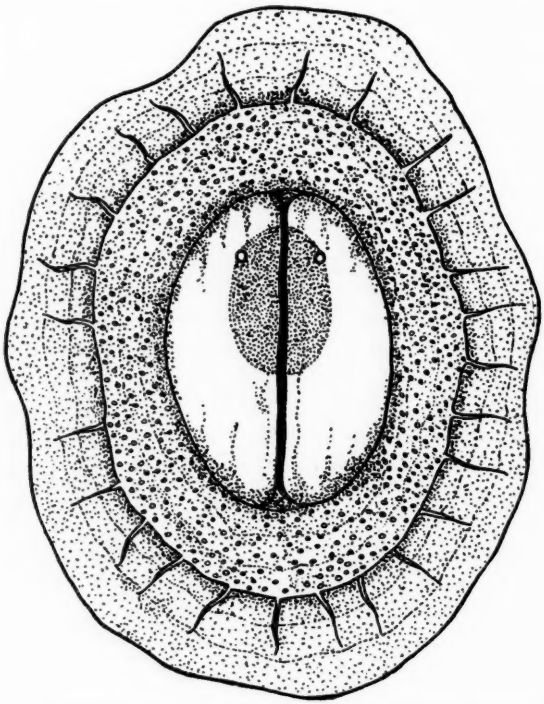

FIG. 1. A settling cypris of *Balanus crenatus* Bruguiere, showing one row of gelatinous spicules and the triple folds of gelatinous material which later is altered and becomes the base of the attached barnacle, thus elevating the organism at right angles to the base of attachment. The darker interior shows the shrunken internal structures of the organism.

while in some cases it was taken in deep water, up to ninety-eight fathoms.

Darwin has described the Balanus group at length, and mentions that he verified his findings by a number of adult specimens of various species. For the verification of the cement apparatus he includes (1853, p. 150) *Balanus crenatus* Bruguiere. Darwin states that after metamorphosis Balanus always secures attachment by a prehensile antenna through which the cement escapes (1853, p. 138). "The central slip, or rather, disc of membrane is 3/400 of an inch in diameter; and this shows the

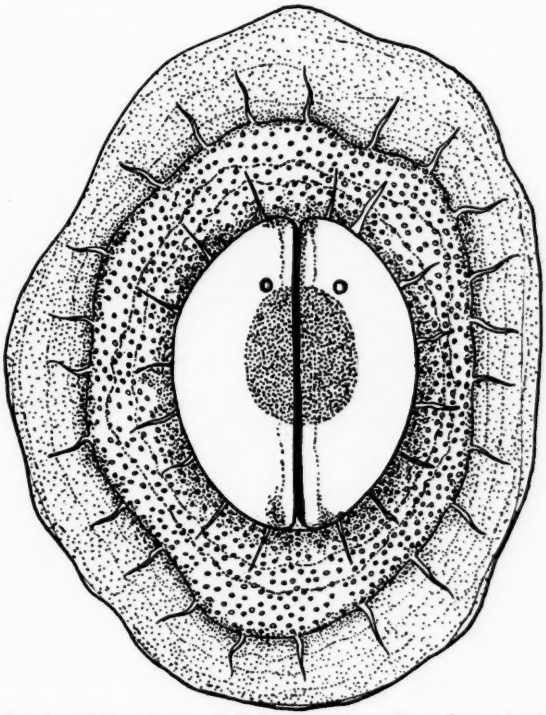

FIG. 2. A settling cypris of *Balanus crenatus* Bruguiere, showing two rows of gelatinous spicules.

basal diameter of the shell immediately after metamorphosis. In the middle of this little disc I saw, in several specimens, the prehensile, pupal antennae. . . . In a full-sized specimen there are from thirty-four to forty cement glands on each side, always corresponding exactly with the number of slips of basal membranes and including the circumferential slips to which the last-formed pair of glands and cement ducts belong."

Hoek (1), in his report of the cement glands of *Lepas species,* differs from Darwin in his findings. He states that the cement ducts are distinctly separated from the ovarian tubules.

Groom (15) spent two years studying the embryological development of several species of barnacles. *Balanus*

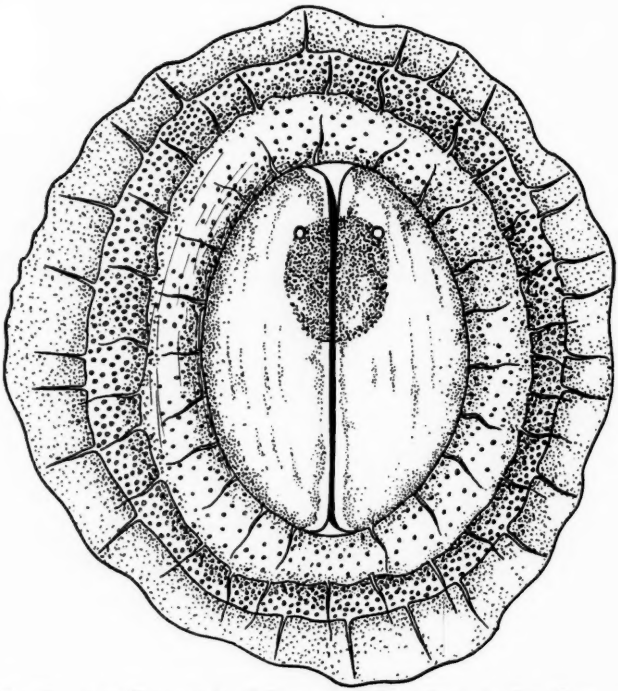

FIG. 3. A settling cypris of *B. crenatus* Bruguiere, showing three rows of gelatinous spicules.

perforatus Bruguiere is mentioned as one of those described, and he found that the early development of the egg of the barnacle was definitely specific for the species, even to the exact number of spines on a cirrus.

DESCRIPTION OF THE *Balanus crenatus* BRUGUIERE CYPRIS FOUND IN THE WATERS OF PUGET SOUND

The experimental Norfolk steel plates (12 x 18 in.) used to secure slime accumulation were suspended from a raft to the depth of twenty feet in the Puget Sound waters near Bremerton, Washington, the U. S. Naval Station, as was also a screened case holding seventeen microscopic glass slides (1 x 3 in.).

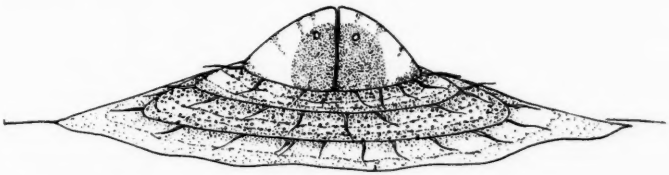

FIG. 4. Cross section of a settling cypris of *B. crenatus* Bruguiere.

The Navy has used the N. S. plates submerged in the Puget Sound waters for several years past in various experiments of anti-fouling paints, etc. Therefore, when the work was started in February, 1924, a few N. S. steel plates which had been left from the previous year's experiment were found to have adult barnacles attached to the submerged plates of the raft. Eggs were found to be present at this time at the sides of the bodies in the mantle cavities.

Nothing new was observed to add to Bray's (3) description of the nauplius, which is an organism of triangular shape, possessing three pairs of setigerous appendages and exhibiting a jerky motion. The nauplius usually moves with its ventral surface upward. It moults several times during a varying space of time (seven to ten days), and is then transformed into the "cypris."

The cypris was found to be a small, elliptical, transparent bivalve organism, 70 x 100 microns. The dorsal edge was wider than the ventral, and one end, the anterior, more blunt than the other, the thoracic. Following the transition stage from nauplius to bivalve, the organism was quiescent and remained on its side. When the muscles were strong enough to contract and permit the opening of the valves, the posterior or dorsal edge widened and the cypris rotated until it rested on the dorsal ridge, thus placing the ventral edge uppermost. The cirri were extruded when the opercular valves opened, and the cypris, propelled by its own power, made slight, jerky, progressive movements. Continued observation

showed that the duration of the motility was variable, limited by a strong tendency to become sessile. The cypris widened along the median line of the dorsal ridge and increased in depth below the valves into a gelatinous ring, and thus became sessile. The upper and lower edge of this ring terminated in a fringe of equidistant gelatinous, hair-like filaments. The lower fringe consisted of about thirty to forty short gelatinous tubules, which aided the settling barnacle in the distribution of its cement from the cement glands and ducts, as a gelatinous suctorial film. The organism could now be pushed along on a flat, smooth surface. With a small instrument the organism could be slightly elevated at either end, thus demonstrating the absence of attachment by an antenna.

The organism raised itself parallel to its base of attachment as the gelatinous material increased. As the gelatinous ring increased in depth and breadth it quickly assumed the form of the calcareous parapet of the adult barnacle.

A few specimens showed some of the hair-like filaments protruding before the organism rotated to its dorsal ridge. Also, some specimens showed a third row of filaments midway between the upper and lower rows on the outer surfaces of the gelatinous mantle. The filaments of the upper and central rows caught gelatinous slime composed of algae, diatoms and bacteria, and held it, apparently to make the food supply more available for the young barnacle. Possibly the fringes provided a means of defense, as the barnacle, at this stage, is very frail and helpless.

OBSERVATION ON THE SETTLEMENT OF CYPRIS OF THE OCEAN BARNACLE, *Balanus glandula* DARWIN, FOUND IN THE TIDE POOLS ALONG THE BEACH AT ALKI POINT OF WEST SEATTLE

The cypris of *Balanus glandula* appeared as a dark, glassy, pitted bivalve, having a dark center, sometimes

pale to dark green, and having the valves shorter and narrower than the other species noted, measuring 34 x 85 microns. The bivalve arrangement and side-resting positions remained the same in the quiescent stage, which was of slightly longer duration, but accompanying the rotation of the bivalve a prehensile antenna was extruded from the blunt end back of the opening of the valves. By this foot the organism was enabled to effect an attachment to a supporting surface, on which it was able to rotate through an angle of 90°. The organism resisted removal and became less glassy in appearance. The dorsal ridges slightly broadened and became posterior, while the thin ventral edge separated, thus permitting the black or dark green cirri to vibrate forward freely. No gelatinous material was secreted on the outside about the organism, which appeared to have a dry, pitted, resistant covering. The opercular valves developed from the valves, and the organism gradually became sessile. The development was slower than in the other species and sometimes hours after the appearance of the parapet the organism appeared to be securely attached only by the pseudo foot. This latter condition probably accounts for the excessive crowding of this species of barnacle on the rocks of arid stretches between tides along the beaches, in its struggle for existence.

Summary

It would appear from Darwin's observations upon adult barnacles and those of the writer during the stage of metamorphosis and the process of attachment of *B. crenatus* Bruguiere that the prehensile antennae of this species were attenuated. Verification for the contention is obtained from the historical data reported by collectors as to the scarcity or absence of the species on arid and rocky beaches, contrasted with its abundant occurrence where submerged by the tide during its first few hours of attachment. The early development of the cement glands may be to overcome the buoyancy of the

water, while the cypri are in their natural habitat; or the ample supply of water may favor the abundant supply of gelatinous material.

References in the literature to the recovery of *B. glandula* Darwin from any stated depth of water are lacking.

Camera lucida drawings were made of the cypris of *B. crenatus* Bruguiere which had been secured to glass slides during their early metamorphosis into the attached barnacle.

Thanks are due to Lieutenant E. W. Sylvester, of the U. S. Navy, for his many courtesies during the experimental work; and to Mr. Henry A. Pilsbry, special curator of the department of Mollusca, Academy of Natural Sciences, Philadelphia, who identified the two species submitted to him from adult barnacles taken from the experimental Norfolk Standard steel plates, and from rocks along the shore of Alki Point; to Professors Trevor Kincaid and John Guberlet, of the zoology department of the University of Washington, for their kindness in reviewing the microscopic specimens and data; to Ernest C. Angst, who made the free-hand drawings of the cypri of *B. crenatus* Bruguiere.

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DOMINANT MUTATIONS OF THE JAPANESE MORNING GLORY

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As is generally the case, dominant mutations are rather rarely found in the Japanese morning glory, *Pharbitis Nil* (Imai 1927c). In this short note, the authors describe two dominant mutations, one that occurred vegetatively and the other seminally under their observation.

Margined-1. A vegetative mutation occurred which produced flowers with completely white margins in one individual of a family (No. 25) of pedigree RI-18. This family was showing segregation for white margins, a character due to the dominant gene margined-1 (Mr1), which is closely linked with contracted. The plant mentioned above originally bore flowers with partially white margins, due to the heterozygous composition Mr1/+; while in its later growth the main vine changed so that it bore flowers with perfectly white margins, the new character appearing in the flowers borne on the upper part of the main stem. Some flowers at the border between the mutant and non-mutant types had mosaic patterns, the margin being partly perfect and partly imperfect, as illustrated in Fig. 1. The quantitative alternation in degree from imperfect to perfect means a genetic change of Mr1/+ to homozygous Mr1, which was definitely proved by the examination of the progenies of the respective parts of the mosaic plant.

In other experiments the authors have noticed two independent cases in which flowers having a self-colored genotype had a small area of white margin. But the white part was so small and limited that such flowers did not give progeny having white margins. Presumably, in such cases also the recessive allelomorph of the gene for the white margin, probably mr1, mutates to the dominant allelomorph (Mr1), though very rarely.

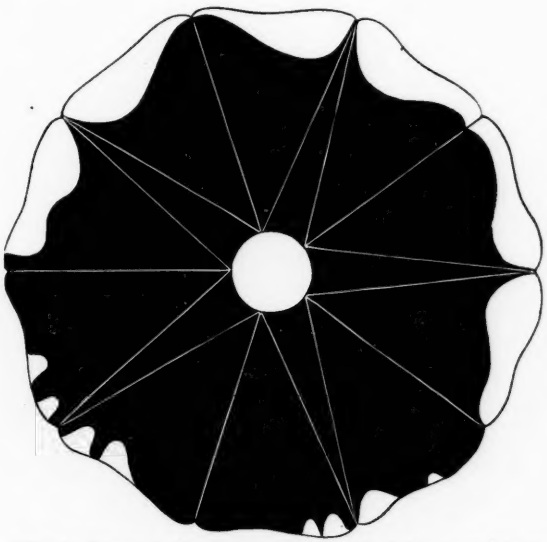

FIG. 1. Mosaic flower, half of the white margin being complete and half imperfect.

The original plant from which our morning glory has been evolved evidently lacked the white margin, that is, it had a self-colored flower. The appearance of the white-margined flowers in the history of this plant under cultivation was noted about three quarters of a century ago. We can see beautiful drawings of specimens having white margins in old illustrated books such as *Asagao-Hanaawase* (1853), *Santoitchô* (1854), etc., before which publications we have no illustrations for the white-margined flowers. The genetics of the white margins is complicated. Five genes, margined-1, margined-2, margined-inhibitor, margined-fluctuator and margined-reducer, have already been detected (Imai 1927a, 1927b). We can not tell in what order these genes appeared in this plant. But the occurrence of the mutation recorded in the present paper suggests the probable method by which margined-1 flower originated from a self-colored morning glory.

White-4. Three white-flower genes, white-1, white-2 and white-3, have been detected in this plant, their genetic behavior to the colored invariably being recessive (Imai 1929). A white flower may have any one of three combinations with respect to stem color and flower-tube color: (1) white tube with green stem, (2) colored tube with green stem and (3) white tube with colored stem. But the fourth combination, colored tube with colored stem, has never been observed. Even in the hybrid progeny of two whites, one having white tube with colored stem and the other colored tube with green stem, the combination of colored tube with colored stem can not be obtained among the white segregates. In such F_2, however, white flowers with white tubes and green stems are produced, being double recessive combination of the two white-flower genes, white-1 and white-2.

In 1926, when the authors were making observation on the flower color of the progeny of plant 361a, it came to their attention that one plant among a total of 109 bore white flowers. This was unexpected in this family, the other plants all bred true to colored flowers. The white flower had a colored tube notwithstanding the fact that its stem was colored—a new combination formerly lacking in the white-flower series. The white corollas of the mutant were wrinkled (Fig. 2), but show fluctuation to the perfect condition. The progenies of the sister plants of this white mutant all produced only colored flowers.

On selfing the white mutant, twenty-five plants were obtained in the subsequent generation; of these nineteen had white and six had colored flowers, which indicated that the white is a simple Mendelian dominant character. The white-flowered segregates were characterized by wrinkled corolla, colored tube and colored stem, as mentioned above. The flower color of the plants, which were segregated as recessives from the white mutant, were dilute purplish brown (Ridgway's "Color Standard and

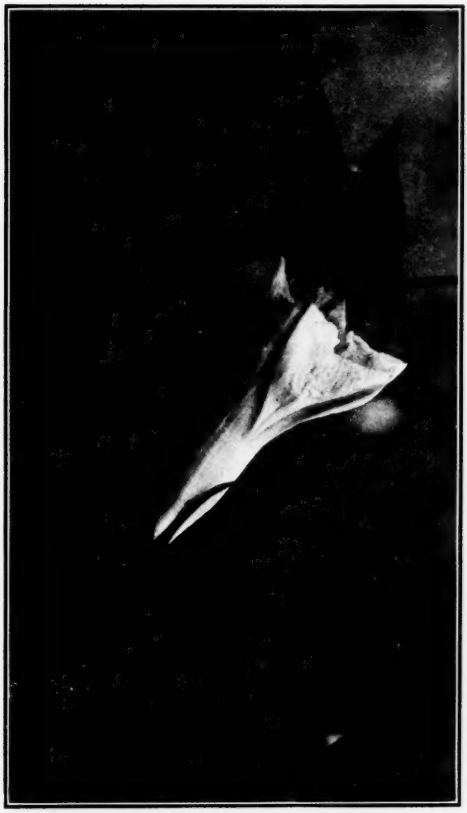

FIG. 2. The white-4 mutant.

Color Nomenclature" (1912), Purplish Vinaceous), a type due to a mutable gene which is furnishing material for a special study. The further data gathered in 1928 proved the simple dominance of the white mutant character, which will be called white-4 (W4), to the colored condition mentioned above. A total of thirteen families which were heterozygous for white showed the segregation of 428 white and 156 colored flowers, where simple expectation is 438 and 146, respectively. Practically,

however, the case is much complicated by the mutable behavior of the gene contained in the segregates; the detailed account will be published later.

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THE GROWTH OF THE LOGGERHEAD TURTLE

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TURTLES, according to common belief, grow very slowly and attain great age. Their unusual length of life has recently been reported on by Babcock (1928) and is well attested by an excellent collection of statistics gathered by Major Flower (1925, p. 978), who has shown that at least four species of turtles of the genus Testudo have indisputable records of a range of life reaching to one hundred years or more. Major Flower concludes that, so far as our present information goes, turtles may be said to live longer than any other vertebrate. Next in point of age to turtles, according to him, are alligators and crocodiles. But great length of life is not characteristic of all reptiles; chameleons, for instance, have never been known to live beyond five years.

Though it must be granted that turtles often reach great age, it is still an open question as to the rapidity of their growth. Louis Agassiz (1857, p. 290), who studied the painted turtle in this particular, concluded that increase in size in this species was very slow and that the females did not begin to lay eggs till they had reached the tenth or eleventh year. He further recorded (1857, p. 417) a case of a marked snapping turtle that had increased its length by only one inch in forty-five years. Such observations support the common opinion that turtles come to maturity only very slowly.

There is, however, much recent evidence to show that turtles may grow with fair rapidity. Mitsukuri (1905, p. 265) in his account of the turtle industry of Japan states that the Japanese snapping turtle, Trionyx, reaches sexual maturity in about six years, when it may weigh 750 grams and have a length of 17.5 centimeters. Specimens

of this turtle with a maximum length of a foot (about 30 centimeters) have been occasionally recorded. These individuals, in the opinion of Mitsukuri, must have been very old. The American diamond-back terrapin, according to Barney (1923, p. 110), may begin to lay eggs when it is only four years old. Coker (1920, p. 176) remarks that this turtle has been known to attain a length of five and a half inches in five years, the maximum observed length being about seven inches. These small turtles must therefore be admitted to grow with considerable rapidity. Nor is this trait limited to such species. Lucas (1922, p. 304), who is apparently quoted by Pellegrin (1926, p. 48), records a large Galapagos turtle that grew in weight from 29 pounds (about 13 kilograms) to 350 pounds (about 160 kilograms) in somewhat less than ten years, a twelvefold increase. These relatively rapid rates of growth in turtles are further assented to in general by Lucas (1922) who in discussing the growth of large animals such as whales declares that these creatures like the larger turtles probably arrive at their mature size in far less time than is generally believed.

Some two years ago I published a record of growth for a loggerhead turtle, *Caretta caretta* (Linn.), in which it was shown that in three years this turtle had changed from its weight at birth, probably 20 grams, to 19 kilograms (42 pounds), an increase that favors the idea of rapid rather than slow growth. Since then through the kindness of Mr. P. R. Stephenson, formerly acting superintendent of the Key West station of the United States Bureau of Fisheries, I have received further records of the growth of the turtle just mentioned as well as records from three other loggerhead turtles. These records, which extend over a period of more than four years, are plotted in Table 1, which also includes my earlier observations. The new records were brought to a close by the accidental escape of the turtles from the pond in which they were confined. It, therefore, seems desirable to publish such observations as are for the moment at hand. I

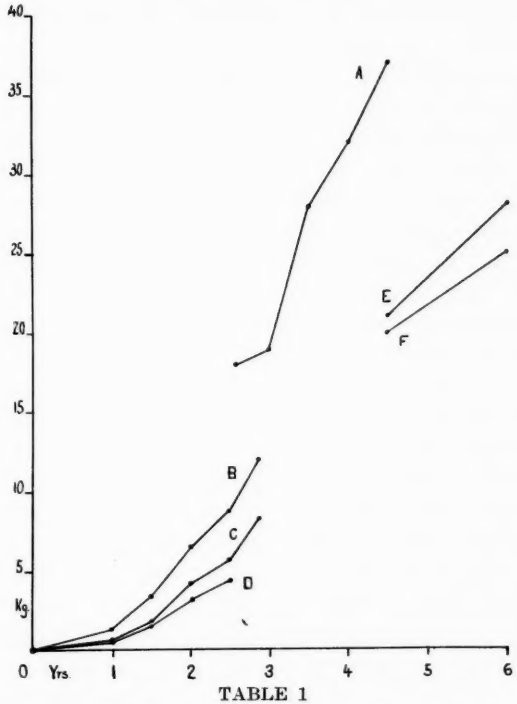

TABLE 1

Growth of six loggerhead turtles, *Caretta caretta* (Linn.), expressed in kilograms and covering a period of six years. Turtle A reported by G. H. Parker from Key West, Florida; turtles B, C and D reported by P. R. Stephenson from Key West, Florida; turtles E and F reported by S. F. Hildebrand and C. Hatsel from Beaufort, North Carolina.

am under obligation to the United States Commissioner of Fisheries, Mr. Henry O'Malley, for permission to publish these results, and for the services rendered by Mr. Stephenson in caring for the turtles and in taking their measurements.

An inspection of Table 1 will show the relative rates of growth of the several loggerhead turtles under consideration. Turtle A, the individual recorded in my first paper (Parker, 1926), though transferred from the aquarium where it was reared to a more open situation, continued

its rapid rate of growth till its last weighing in January, 1928, when at an age of four and a half years it had reached the weight of 37 kilograms. Since adult loggerhead turtles are usually assumed to weigh about 300 pounds (Babcock, 1919, p. 346), or roughly 135 kilograms, turtle A may be said to have accomplished a little more than the first quarter of its growth in four and a half years. The rate, therefore, was relatively rapid. How long it would take such a turtle to reach 135 kilograms can not, however, be stated.

The three remaining turtles, B, C and D, escaped from their pool between the ages of two and three years, D a few months earlier than B and C. Their rates of growth were decidedly lower than that of turtle A. At two and a half years of age turtle B, the most rapidly growing individual of the three, had reached a weight of only 8.5 kilograms, or less than half the weight of A, 18 kilograms, at the same age. Similarly turtle D, the slowest of the three, was at two and a half years less than one fourth the weight of A at that age. Thus all three turtles were growing at a lower rate than A. Their rates, however, fall fairly well in line with the probable rates of the two loggerhead turtles whose weights have recently been reported by Hildebrand and Hatsel (1927). These turtles were found to weigh at four and a half years 45 pounds (about 20 kilograms) and 47 pounds (about 21 kilograms) each and at approximately six years 55 pounds (about 25 kilograms) and 61 pounds (about 28 kilograms) each. If these records are compared with the others in Table 1 it is evident that the rates of growth of these two turtles must have been much less than that of turtle A and in rough agreement with those of turtles B, C and D. Judging from the table as a whole it is probable that the lower rates of growth are more nearly normal for loggerhead turtles than the rate for turtle A, but, however this may be, even these lower rates are relatively rapid and favor the view that large turtles like small ones may grow rapidly.

The curves plotted in Table 1, especially those for turtles A, B, C and D, suggest the beginning of the typical sigmoid curve of growth. Since the longest of these curves, that for turtle A, represents only the first quarter of this turtle's increase in weight the interpretation just suggested is not unreasonable. From the standpoint of growth, records of weight covering the whole life of a given turtle would be very much worth while. Whether the period from birth to sexual maturity in turtles is one fifth the total length of life of the animal, as it is claimed to be for many mammals, is quite unknown. On the whole it seems improbable that the growth of a turtle is as definitely subject to rule as that of a mammal.

Growth in general is more truthfully expressed by increase of weight than by any other single aspect of an animal. Nevertheless, changes in linear relations are not without interest. The carapace of a newly hatched loggerhead turtle measures approximately 4.8 centimeters in length by 3.5 centimeters in width. When turtle A after four and a half years of growth had reached the weight of 37 kilograms its carapace measured 63 centimeters in length and 59 centimeters in width, a linear increase of roughly fifteen fold.

It is clear from the evidence presented in this paper that turtles, even large ones, may reach maturity at a comparatively rapid rate, and that the opinion commonly entertained of their great slowness of growth is scarcely justifiable. Without doubt their increase in late life is very slow and this may have given occasion for the belief in a low rate of growth in general. Such rates, however, do not apply to their early stages. These stages are characterized by a reasonably rapid increase. One aspect of this process, however, must be kept in mind. Their early growth is subject now and then to temporary checks. This feature appears clearly in some of Pearse's records (1923) on the western painted turtle, whose variations in this respect are often very considerable.

These variations, which are familiar to all those who have undertaken to breed turtles (Coker, 1920, p. 176; Barney, 1923; Hildebrand and Hatsel, 1927, p. 377), doubtless reflect fluctuations in environmental conditions in which not only simple food supply but vitamins and the like probably play an important part (Pearse, Lepkovsky, and Hintze, 1925; Parker, 1926).

SUMMARY

(1) The loggerhead turtle, *Caretta caretta* (Linn.), on hatching weighs about 20 grams and its carapace measures approximately 4.8 centimeters in length by 3.5 centimeters in width.

(2) An individual reared in confinement at Key West, Florida, when four and a half years old, weighed 37 kilograms, or a little over one fourth the average adult weight. Its carapace measured 63 centimeters in length and 59 centimeters in width. Its rate of growth was several times more rapid than that shown by three other loggerhead turtles reared under almost similar conditions. Even these lower rates, however, indicate that turtles grow to maturity much more rapidly than is generally supposed.

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SHORTER ARTICLES AND DISCUSSION

A GENERAL SCHEME FOR THE ORIGIN OF MUTATIONS

EXPERIMENTS on the induction of mutations by means of X-rays, begun with the brilliant work of Muller and repeated with the same success by other workers including those of our laboratory, have shown a relationship between several types of mutation. In these experiments there has been obtained not only a great increase in the frequency of different point mutations (visible, semilethal and lethal) but perhaps in a greater degree an increase in the frequency of more radical changes in the structure of chromosomes—such as inversions, translocations, etc. The quantity of inversions and translocations obtained in our laboratory as well as by Muller and Weinstein perhaps exceeded in some experiments the number of simple transgenations.

It is, therefore, scarcely possible to doubt that there is something general in all these mutational phenomena. This idea has been expressed before. Thus Muller has already pointed out the possibility of considering deficiency and duplication as part of one phenomenon, namely, translocation. If in translocation a part of one chromosome attaches itself to another, then depending on circumstances this phenomenon may be described in several different ways. Thus if only those individuals are found viable which carry simultaneously both a chromosome with a broken off fragment as well as the one to which the fragment has become attached, this may be described as a *translocation* in the proper sense of the word. If also those individuals are found viable which have a translocated section but which lack the chromosome from which the translocated piece was taken, it may happen that the chromosome with the broken off fragment will disappear and we shall have for investigation only those individuals containing a translocation on one chromosome while the other chromosomes are normal. In this case we shall have a *duplication.*

A third case is possible, when a translocation is lost and we have only a chromosome left with a broken off fragment. This case will be described as a *deficiency.*

This scheme uniting translocations, duplications and deficiencies seems to be most probable. From our point of view, there are no definite limits differentiating deficiencies from other muta-

tions. A series of deficiencies such as dominant mutation of the Notch type, recessive lethal mutations, semilethals and visible mutations shows such a quantity of gradual changes that any border line made here would be quite artificial. The analysis of what we know of different mutations of Drosophila and of multiple allelomorphs persuades us in the idea that the deficiencies and lethals differ from visible mutations principally by the size of the mutated section of the chromosome. That is to say that not only the lethal but the visible recessive mutations are only "small deficiencies."

Considering visible and lethal mutations in such a way we may derive a unique scheme for a series of mutational phenomena. For a long time the phenomena of "*inversion*" could not be included in such a scheme, although now the connection of inversions with other types of mutations is apparent in two points, first that the X-rays give the same increase of frequency in lethals as well as in inversions; and second that the inversions are accompanied by the simultaneous appearance of lethals.

At the present moment we may propose a simple scheme which unites satisfactorily all known types of mutations, except changes in the number of chromosomes the mechanism of which can be easily understood. Our scheme rests on the assumption that chromosomes coming into contact in different ways may in one way or another get attached to each other. If later something makes these attached chromosomes break away from each other, then the rupture may be followed by the removal of some part of the chromosome.

This assumption allows us to make schemes for the following mutations:

(1) *Translocation* from one chromosome to another (Fig. 1).

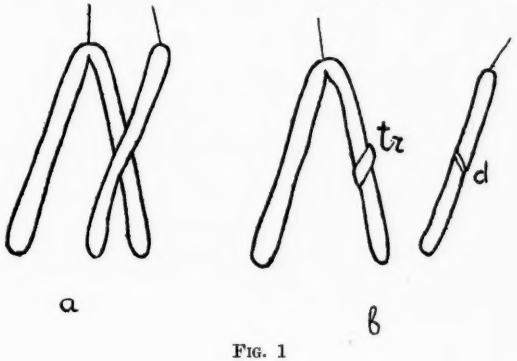

FIG. 1

If a chromosome joins another at some one point, then after rupture of this attachment a fragment of the first chromosome may remain in the second chromosome and in the first chromosome will occur a corresponding deficiency.

Depending on the size and locus of the broken off fragment of the first chromosome there will arise in it either a deficiency, a lethal or a visible mutation. If the translocation retains without loss the whole fragment which is broken off from the first chromosome, then the phenotype of the fly may remain unchanged.

A change will take place only in cases when the attachment of the translocation to the second chromosome interferes with the activity of those genes which are located at the locus of attachment of the translocation. If in the rupture of the attached chromosomes a part of the translocation disappears, then the deficiency will be longer than the remaining part of the translocation and the individuals carrying both changed chromosomes may reveal some lethal or visible character.

(2) *Translocation* within one chromosome (Fig. 2).

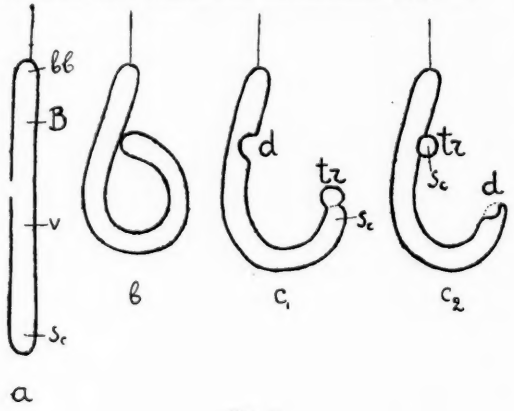

FIG. 2

The scheme of this phenomenon only slightly differs from the above-described case but it gives an immediate transition to *inversion.*

(3) *Inversion* (Fig. 3).

If the rupture of the chromosome happens after attachment as is shown in this scheme then a part of the chromosome, *e.g.*, from s_c to *B* loci, will be inverted.

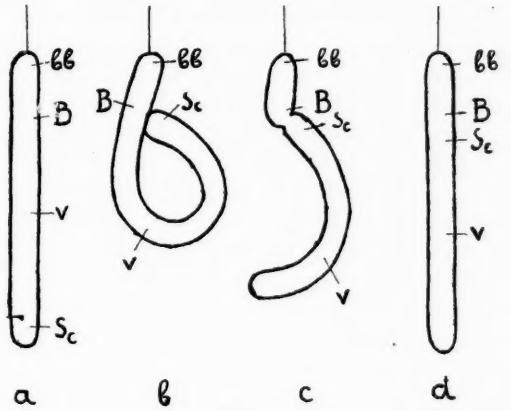

FIG. 3

(4) *Inversion* of the middle section of chromosome (Fig. 4).

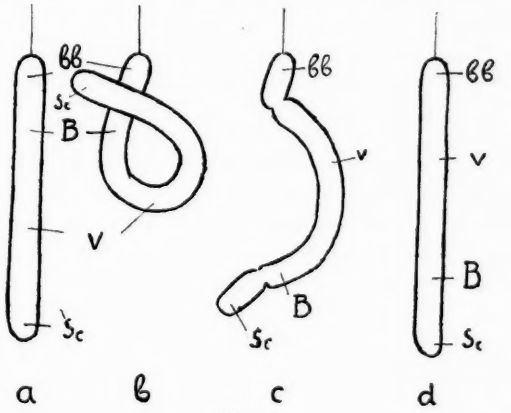

FIG. 4

In this scheme only the middle section of the chromosome will be found inverted. The ends *bb* and s_c will keep their previous positions. If the secondary rupture of the chromosome is followed by the loss of a fragment of chromosome the inversion will be accompanied by lethal effects at one or both ends of the inversion.

The point of view here developed which considers all types of mutations as a result of similar processes demands of course that visible and lethal mutations and translocations must take place

simultaneously. The investigation of this question may now be undertaken as we have now such a powerful tool for obtaining mutations in the X-ray. But it must be kept in view that the discovery of translocations is possible only under certain favorable conditions, either when the translocation covers the lethal or visible mutation and therefore the individuals carrying both deficiency and translocation are at the same time normal and viable and the individuals which have lost the translocation are nonviable or display a visible character; or when the chromosome itself carrying translocation produces a visible or lethal effect. If the translocation does not quite cover deficiency, the presence of such a translocation may not be noticed, since the lethal effect of deficiency will appear alike both in the presence and absence of translocation. In this case the presence of translocation can be discovered only under other favorable conditions, such as, for instance, if the translocation changes the amount of crossing over in the region of its attachment. To prove that translocation takes place in every case of transgenation it is necessary to have an extensive series of rather difficult experiments and unfortunately it may be predicted beforehand that the occurrence of translocation will not always be detectable.

However, this hypothesis may be tested in an indirect way. For example, we can foresee that the translocations should show some sequence in the loci of their occurrence. Thus translocation within a chromosome most likely takes place from one end to the other, but not within one small region. The mean distance within which translocation may occur within a chromosome must bear a certain relation to the size of inversion taking place in these chromosomes, etc. The hypothesis is here presented since it allows certain deductions which may be tested by experiment.

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THE EFFECT OF DIFFERENT SECTIONS OF THE X-CHROMOSOME UPON BAR EYE IN *DROSOPHILA MELANOGASTER*

LINKAGE data from breeding experiments indicate that the genes with reference to their main effects are perhaps distributed at random on the chromosome. The genes also have less well-known secondary effects. In regard to these secondary effects,

on the basis of knowledge obtained as a by-product of experiments carried out for other purposes, it is tentatively held that the genes as plus and minus modifiers are likewise distributed at random in the germplasm. There is some evidence of an indirect sort which indicates that the plus and minus modifiers in combination are merely additive, although it is recognized that the situation is a complex one.

It is due mainly to the analysis made by Morgan and his coworkers that the germplasm of at least one organism, *Drosophila melanogaster*, has been mapped with a sufficient degree of completeness to allow, for the first time, a study of the effects of various combinations of the genes upon a particular characteristic, and so to obtain data from which an analysis of their functional relationship may be made. An excellent character upon which to make such studies is Bar eye, not only because it is readily modified by genetic and environmental factors, and a quantitative measure obtained of such modification, but also because Zeleny's low Bar, rendered homozygous for modifiers of eye size by long-continued selection, makes a most excellent base of reference for such work.

By suitable matings, using a multiple recessive stock of the X-chromosome, (scute, echinus, crossveinless, cut, vermilion, garnet, forked), and a low selected forked Bar stock, the fifteen combinations indicated in the accompanying table were formed. The flies were raised at 25° C. and the facet counts of the dorsal and ventral lobes were recorded separately.

The facet counts of the dorsal and ventral lobes in any given stock conform to the expression

$$y = bx^k$$

in which x and y are the number of facets in dorsal and ventral lobes respectively, b gives the ratio of the size of the ventral to dorsal lobe at the time when the two lobes begin forming facets logarithmically in the ratio indicated by k. It should perhaps be pointed out that the value of b for any stock in terms of facets may not be given since it is unknown whether the effective period of facet formation in the larva begins with a spurt or a lag period. Likewise, k gives no indication of the absolute logarithmic rate of facet formation in the dorsal and ventral lobes of the eye. However, when $k = 1$, the logarithmic rate of formation is the same in the two lobes; when $k = 1.50$, the rate of formation

in the ventral lobe is 1.5 times that of the dorsal lobe; when $k = 2/3$, the rate of formation in the dorsal lobe is 1.5 times that of the ventral lobe.

In the accompanying table, which is for males only, and based on about 1,400 flies, are given the mean facet numbers of the entire eye, the differences in the means referred to forked Bar, and the numerical values of b and k in the above equation, calculated by the method of least squares.

♂ ♂	Mean	Difference with *fB*	*b*	*k*
sc + + + + + f B	88.29 ± 1.07	+ 0.26 ± 2.09	0.22	1.24
+ ec + + + + f B	92.89 ± 1.41	+ 4.87 ± 2.28	1.05	0.85
+ + cv + + + f B	111.97 ± 2.73	+ 23.94 ± 3.26	3.18	0.61
+ + + ct + + f B	72.75 ± 0.83	− 15.28 ± 1.97	1.24	0.80
+ + + + v + f B	106.54 ± 2.36	+ 18.51 ± 2.96	0.24	1.17
+ + + + + g f B	63.71 ± 0.61	− 24.32 ± 1.89	1.20	0.79
+ + + + + + f B	88.03 ± 1.79	0.00	0.37	1.11
sc + + + + + f B	88.29 ± 1.07	+ 0.26 ± 2.09	0.22	1.24
sc ec + + + + f B	93.57 ± 1.21	+ 5.54 ± 2.16	0.50	1.02
sc ec cv + + + f B	111.51 ± 1.26	+ 23.48 ± 2.19	0.89	0.91
sc ec cv ct + + f B	140.21 ± 2.68	+ 52.18 ± 3.22	2.28	0.71
sc ec cv ct v + f B	146.09 ± 3.11	+ 58.06 ± 3.59	2.14	0.73
sc ec cv ct v g f B	90.76 ± 3.78	+ 2.73 ± 4.18	1.95	0.75
+ + + + + g f B	63.71 ± 0.61	− 24.32 ± 1.89	1.20	0.79
+ + + + v g f B	70.89 ± 1.01	− 17.14 ± 2.06	0.49	1.07
+ + + ct v g f B	71.95 ± 0.85	− 16.08 ± 1.98	0.90	0.88
+ + cv ct v g f B	80.38 ± 1.38	− 7.65 ± 2.26	2.49	0.66
sc ec cv ct v g f B	90.76 ± 3.78	+ 2.73 ± 4.18	1.95	0.75

It is seen that, when the sections of the Xple chromosome designated by the mutant genes are added singly to forked Bar, cut and garnet are pronounced minus modifiers and the others are plus modifiers, crossveinless and vermillion notably so.

The middle section of the table shows the results of adding the mutant genes cumulatively in the left-right direction on the chromosome. The eye becomes progressively larger until garnet is added. With the addition of garnet, that is in Xple Bar, the eye is not significantly larger than in forked Bar.

Beginning with the left end of the chromosome, certain further comparisons may be pointed out. The effect of scute and echinus acting together may be considered as the additive effect of scute and echinus taken singly, when the comparison is made on the

basis of average facet number. The value of *k* when scute and echinus act together is approximately the average of scute and echinus when taken singly. Although scute, echinus and crossveinless are plus modifiers of facet number, the cumulative effect of the three in combination (*sc ec cv* + + + *f B*) is perhaps no greater than that of crossveinless acting alone. The section of the chromosome bearing crossveinless may be said to dominate in some sense the section of chromosome to its left, a phenomenon which is somewhat like yet different from either dominance or epistasis. When these same combinations are compared with reference to the values of *k*, it is seen that scute and echinus do play a rôle, since the value of *k* for the three acting together is approximately the mean of the three values of *k* for the same genes taken singly.

Cut alone is a fairly strong minus modifier of facet number, but in the combination *sc ec cv ct* + + *f B*, it becomes a strong plus modifier. This is in some respects like the case of sepia, which is a darkener of eye color when the wild type is the base of reference, but a diluter in combination with eosin. But in regard to *k* in the combination under discussion, crossveinless seems to dominate scute and echinus, and the value of *k* becomes practically the average for crossveinless and cut taken singly. Vermilion in the combination *sc ec cv ct v* + *f B* is a plus modifier, but does not have the same effect as when acting alone, as if cut were partially manifesting itself as a minus modifier. The value of *k* is but slightly modified compared to the previous combination. When garnet (which alone is a strong minus modifier) is added, that is in *sc ec cv ct v g f B* the number of facets is not significantly different from forked Bar, but that there is a difference in the facet-producing reaction in the larvae may be inferred from the values of *b* and *k*.

When the genes are added cumulatively in the right-left direction on the chromosome, (3^d section of the table), the eye is smaller than in forked Bar, but becomes progressively larger as more of the Xple chromosome is added, finally reaching a size slightly larger than that of forked Bar. There is no significant difference in mean facet number between + + + *ct v g f B* and + + + + *v g f B*, indicating that here also cut is a weak gene, but that it does play a rôle may be seen in the values of *b* and *k*. Crossveinless again shows itself as a powerful depressor of the value of *k*.

The biological meanings of the terms in the above equation have already been indicated. It is furthermore of interest that there is a definite regularity in the relation between the values of b and k. As k increases in arithmetical progression, b decreases in geometrical progression, that is, they conform to the equation

$$b = Be^{-rk}$$

in which b and k have the meanings already indicated, r is the rate of decrease of b per unit change in k. By estimation from the graph, b shows an exponential decrease of about 44 per cent. for each 0.1 increase in value of k. B in the above equation may be designated the value of b when k is infinitely close to zero. Its value is about 47.0. No detailed biological meaning can be given at present to this uniformity in the covariation of b and k. In a general way, however, it indicates that the dorsal and ventral lobes of the eye are in equilibrium and furthermore that the effective period of facet production does not vary independently of the entire system of which it is a part, namely the larva as a whole. In other words, the character of the effective period of facet production is in some sense under the control of the whole organism.

In conclusion, the data demonstrate a functional regularity of the genes in different sections of the X-chromosome in their effects upon facet number that is not predictable from their effects when taken singly.

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POISONOUS FISHES IN SAMOA

IN 1902, the writer, being engaged in the investigation of the fishes of Samoa, made a report to the commandant at the U. S. Naval Station of Tutuila, (then) Captain Uriel Sebree, setting forth the state of our knowledge of the different species in relation to their poisonous qualities. This report was printed for the use of the people in Tutuila and was accompanied by a series of colored sketches by me of the fishes to be avoided.

I wish to place this report on record for the use of future investigators. The nature of the poison producing the disease of Ciguatera deserves much more attention than it has received before.

Most of the information contained in this report was gathered in the harbor of Apia, island of Upolu.

A: Poisonous Fishes in Samoa

(*Report of Investigation in 1902*)

United States Naval Station, Tutuila

August 9, 1902

Captain Uriel Sebree, U. S. N.,

Governor of Tutuila.

Dear Sir:

Permit me to make an informal report on our investigation of the fishes of the Samoan Islands.

With the aid of Professor Vernon L. Kellogg and Mr. Michitaro Sindo, I have spent seven weeks in the examination of the fishes of Samoa, under the direction of the U. S. Fish Commission, for the information of the president and the government of the United States.

We have found in Samoa 610 different kinds of fishes, a number not surpassed in any other part of the world, and approached, so far as known, in Java only.

Of this number, about one half are small fishes living in crevices and pools of the coral reefs; some of these never exceed half an inch in length. Of the others, most species are good for food and the bays of Tutuila have an unusually large number of good food-fishes.

Fish are sometimes poisonous in these waters. There are three causes of this:

(1) When fish are allowed to lie six hours or more, or for a less time in a wet heap, the flesh becomes tainted, and the contents of the stomach soak into the flesh. Tainted fish produce sickness—vomiting and indigestion—a disease called Ichthyosis. In this respect, one kind may be as bad as another, and the remedy is to dress fish as soon as possible, and to eat them soon after they have been caught. This disease rarely lasts long, and the best treatment is to produce vomiting.

(2) Sometimes fish themselves eat poisonous growths in the sea, thus killing themselves and making their flesh poisonous. There seems to be no such poisonous fish-food in the harbors of Samoa, but it is reported that they sometimes come up from the deep sea after storms.

(3) Certain kinds of fish are sometimes or always poisonous on account of a bitter substance, called an alkaloid, and somewhat like strychnine, which is developed in the flesh. These produce a violent nervous attack called Ciguatera, which is sometimes fatal. The first remedy is to produce vomiting.

The principal fishes in Tutuila which may produce Ciguatera are, so far as known, the following:

(1) The Mumea, a large deep red fish with a long body and a large mouth—a kind of snapper. This is said to be always poisonous (*Lutianus bohar*).

(2) The Taiva Uliuli, a smaller kind of snapper, green in color, with a small black spot on each side of the body (*Lutianus monostigma*).

(3) The Filoa, a very long-nosed snapper, is very often poisonous in Upolu (*Lethrinella miniata*).

(4) The Ataata, a kind of grouper, almost black and covered with small spots of deep blue. This is often poisonous in Tutuila (*Epinephelus merra*).

(5) The Alogo, a lancet-fish, or fish with a sharp knife of bone on the side of the tail, with body covered with bright stripes of blue and orange (*Acanthurus lineatus*).

(6) The trigger fishes, called Sumu, are poisonous in some places, but are said to be always safe in Samoa (*Balistes*).

(7) The file fishes, called Aleva, are regarded as poisonous; they are thin and poor (*Monacanthus*).

(8) All the puffers, or globe fishes, called Sue, and the porcupine-fish, Tautu, are likely to be poisonous—sometimes very poisonous (*Tetraodon, Diodon*). The venom lies in the ovary.

(9) The trunk fishes, Moamoa, are said to be always safe in Samoa (*Ostracion*).

(10) The large sea eels, or morays, called Pusi, *Gymnothorax*, are very often poisonous—some of them always so. The fresh-water eels, called Tuga (*Anguilla*), are always good.

FISHES THAT ARE ALWAYS GOOD TO EAT

(1) All silvery fishes of the bay or rivers are good. Among these are the Safole, Sesele, Anaanalagi, Anae, Ana, Afa, Atule, Ula, Malauli Lupo, Ga, Atualo, 'Ava'ava, Ali, Lalafutu, Ise, Ta'uleia, Gatasami, Malolo, Uisila, Matu, Iasina Umiumia. The Ulua and Malauli (*Caranx*) are especially valuable for their excellence, the Atule (*Trachurops*) for its abundance.

(2) The Bonito and other free-swimming fishes of the deep water, with red flesh, are good.

(3) The parrot-fishes, red, brown, blue and green, known as Fuga, Laea, Galo, etc., are always safe (*Scarus*).

(4) The dark green, blue and variegated fishes, known as Sugale, are always safe (*Thalassoma*, etc.).

(5) The rough-scaled red fishes of many kinds, known as Malau, are always good (*Holocentrus*, etc.).

(6) The little butterfly fishes, known as Tifitifi, are always free from poison (*Chaetodon*).

(7) The green, red or yellow snappers called Tamala, Mataeleele and Malai, are mostly good fishes (*Lutianus*).

(8) The goat-fishes, Matamu, Manifi, Motai and Moaga, are not poisonous (*Upeneus*). In one species of goat-fish, *Upeneoides*, the brain is at times noxious, producing on men and cats a temporary delirium (in Hawaii).

(9) The fishes with poisonous spines, dangerous to touch, Nofu, Sausaulele, Laotale, are always good to eat (*Scorpaena*, etc.).

(10) Sharks, Malie, are coarse-fleshed, and should be eaten sparingly, if at all (*Carcharias*).

(11) The flounder, Ali, is always safe.

(12) The octopus, Fee, is good.

There are about 175 species of fishes in the Harbor of Pago Pago large enough for food and perfectly safe to eat.

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